FINAL

Report on Carcinogens Background Document for

Dyes Metabolized to 3,3′-Dimethylbenzidine

Meeting of the
NTP Board of Scientific Counselors
Report on Carcinogens Subcommittee

Prepared for the:
U.S. Department of Health and Human Services
Public Health Service
National Toxicology Program
Research Triangle Park, NC 27709

Prepared by:
Technology Planning and Management Corporation
Canterbury Hall, Suite 310
4815 Emperor Blvd
Durham, NC 27703
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Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

US 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.

Summary Statement

Dyes Metabolized to 3,3'-Dimethylbenzidine (3,3'-Dimethylbenzidine Dye Class)

Carcinogenicity

3,3'-Dimethylbenzidine-based dyes that are metabolized to 3,3'-dimethylbenzidine are *reasonably anticipated to be human carcinogens* based on the facts that 3,3'-dimethylbenzidine is carcinogenic in male and female rats (IARC 1972; NTP 1991b, 1998) and that metabolism of 3,3'-dimethylbenzidine-based dyes to release free 3,3'-dimethylbenzidine is a generalized phenomenon, occurring in all species studied (Lynn *et al*. 1980; Bowman *et al*. 1982). Additional evidence of the carcinogenicity of this dye class is the fact that a representative 3,3'-dimethylbenzidine-based dye, C.I. Acid Red 114, is carcinogenic in male and female rats (NTP 1991a). Further, the pattern of tumors observed with C.I. Acid Red 114 (NTP 1991a) and 3,3'-dimethylbenzidine (NTP 1991b) is similar to that observed with the structurally similar chemical 3,3'-dimethoxybenzidine (NTP 1992) and the 3,3'-dimethoxybenzidine-based dye C.I. Direct Blue 15 (NTP 1992). Most notably, each of these four chemicals induces increased incidences of tumors in skin, Zymbal gland, liver, oral cavity, gastrointestinal tract, preputial gland of male rats, and clitoral gland of female rats, among other tissue sites.

No adequate human studies of the relationship between exposure to 3,3′-dimethylbenzidine-based dyes and human cancer have been reported.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

3,3'-Dimethylbenzidine is structurally similar to benzidine, a known human carcinogen (IARC 1972, 1979, 1982, and 1987; NTP 1997, 1998) and 3,3′-dimethoxybenzidine, which is reasonably anticipated to be a human carcinogen (IARC 1974; NTP 1992, 1998). Like benzidine and 3,3'-dimethoxybenzidine, 3,3'-dimethylbenzidine is used as a base chemical from which many dyes are synthesized. These dyes are synthesized by linking of various chromophores to the base chemicals via azo linkages. Regardless of the chromophore(s) involved, the azo linkages of 3,3'-dimethylbenzidine-based dyes are chemically equivalent and are readily cleaved by chemical or enzymatic reduction to form free 3,3'-dimethylbenzidine and the chromophore(s). Reductive cleavage of 3,3'-dimethylbenzidine-based dyes to yield free 3,3'-dimethylbenzidine is catalyzed by a number of bacteria, including *Escherichia coli*, found in the human gastrointestinal tract (Cerniglia *et al*. 1982, Morgan *et al.* 1994). Reductive cleavage of 3,3'-dimethylbenzidine-based dyes to 3,3'-dimethylbenzidine also was shown in studies with rats, dogs, and hamsters (Lynn *et al*. 1980, Bowman *et al*. 1983, Nony *et al*. 1983). Metabolism of the dyes to free 3,3'-dimethylbenzidine in animals is thought to be mediated primarily by bacteria in the gastrointestinal tract (Cerniglia *et al.* 1982, Morgan *et al*. 1994). 3,3'-Dimethylbenzidine-based dyes are mutagenic in bacteria when tested with metabolic activation and an azo-reductive preincubation protocol (NTP 1991a). It is

assumed that the reductive system results in the formation of 3,3'-dimethylbenzidine, a known bacterial mutagen (Haworth *et al.* 1983).

No information exists to suggest that the mechanism of carcinogenesis of these substances operating in rats would not also operate in humans.

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1 Introduction

Dyes metabolized to 3,3'-dimethylbenzidine (dimethylbenzidine dyes as a class) were nominated for listing in the Report on Carcinogens (RoC) by the National Institute of Environmental Health Sciences (NIEHS) RoC Review Group (RG1) based on the current RoC listing of the parent compound 3,3′-dimethylbenzidine (DMB) as *reasonably anticipated to be a human carcinogen* and the fact that the azo linkages of DMB-based dyes are chemically equivalent and are readily cleaved by chemical or enzymatic reduction to form free DMB and the chromophore(s).

1.1 Chemical Identification

Dyes are a large and diverse group of organic compounds, many of them water-soluble, that have various applications for coloring numerous products. Dye molecules are colored because they absorb and reflect light. Most dyes in use today are synthetic organic compounds.

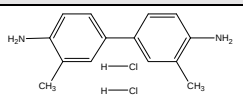
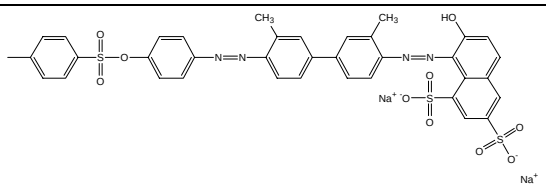
Dyes may be classified according to their chemical structure or their method of application. DMB-based dyes contain DMB attached to other substituents by diazo linkages. The dyes evaluated in this report are examples from the class of DMB-based dyes that have been studied for their potentially carcinogenic properties.

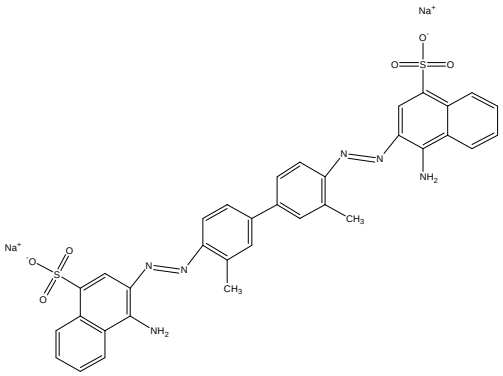
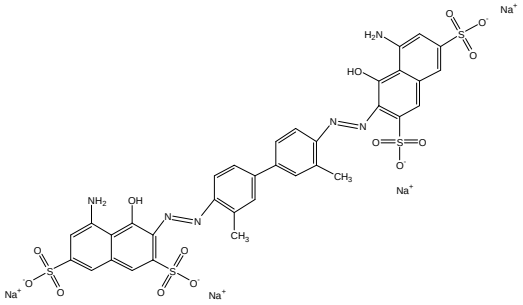
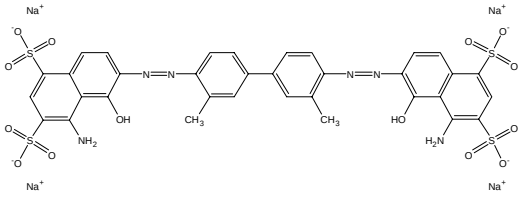
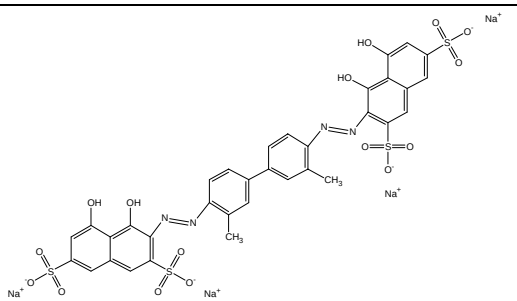
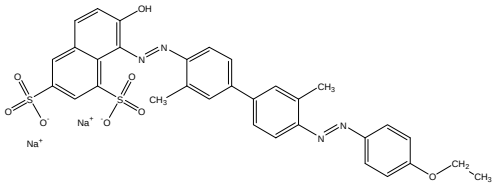
DMB ($C_{14}H_{16}N_2$, mol wt 212.29, CASRN 119-93-7) is a methylated congener of benzidine and also is known by the following names (Chemfinder 1999):

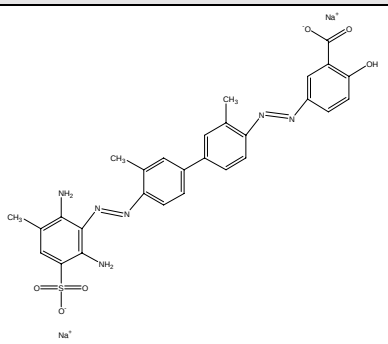
ortho tolidine
fast dark blue base R
3,3'-dimethylbiphenyl-4,4'-diamine
dimethyl benzidine
4,4'-bi-*o*-toluidine
3,3'-dimethyl-4,4'-biphenyldiamine
bianisidine
C.I. 37230
C.I. azoic diazo component 113
3,3'-dimethyl-1,1'-biphenyl-4,4'-diamine
tolidine
3,3'-tolidine
4,4'-diamino-3,3'-dimethylbiphenyl
diaminoditolyl
o,o'-tolidine

The dyes discussed in this report are limited to those containing the DMB moiety and which, upon metabolism, release free DMB. DMB-based dyes for which carcinogenesis and mechanistic studies have been reported in the literature are summarized in Table 1-1.

Table 1-1. Examples of some DMB-based dyes

Dye name and formula	CASRN	Mol wt	Structure
DMB-2HCl $C_{14}H_{18}Cl_2N_2$	612-82-8	285.22	
C.I. Acid Red 114 C.I. 23635 $C_{37}H_{28}N_4O_{10}S_3Na_2$	6459-94-5	830.81	

Dye name and formula	CASRN	Mol wt	Structure
C.I. Direct Red 2 C.I. 23500 $C_{34}H_{26}N_6O_6S_2Na_2$	992-59-6	724.72	
Trypan blue C.I. 23850 $C_{34}H_{24}N_6O_{14}S_4Na_4$	72-57-1	960.79	
Evan's blue C.I. 23860 $C_{34}H_{24}N_6O_{14}S_4Na_4$	314-13-6	960.79	
C. I. Direct Blue 25 $C_{34}H_{22}N_4O_{16}S_4Na_4$	2150-54-1	962.76	
C.I. Direct Red 39 C.I. 23630 $C_{32}H_{26}N_4O_8S_2Na_2$	6358-29-8	704.68	

Dye name and formula	CASRN	Mol wt	Structure
C.I. Direct Orange (disodium salt) $C_{28}H_{24}N_6O_6SNa_2$	6637-88-3	618.57	

Source: (Chemfinder 1999)

1.2 Physical-chemical properties

The chemical and physical properties of the DMB-based dyes listed in Table 1-1 are summarized in Table 1-2. Table 1-3 summarizes the physical and chemical properties of DMB.

Table 1-2. Physical and chemical properties of some DMB-based dyes

Dye name and formula	Color and physical state	Melting Point (°C)	Water solubility (g/100mL)
DMB-2HCl $C_{14}H_{18}Cl_2N_2$	light tan powder	NA	1 – 5 at 22°C
C.I. acid red 114 $C_{37}H_{28}N_4O_{10}S_3Na_2$	dark red powder	250 – 300[a]	< 0.01 at 22.5°C
C.I. Direct Red 2 $C_{34}H_{26}N_6O_6S_2\ Na_2$	brown powder	290	< 0.1 at 16°C
Trypan blue $C_{34}H_{24}N_6O_{14}S_4\ Na_4$	bluish-gray powder	> 300	0.1 – 1 at 20°C
Evan's blue $C_{34}H_{24}N_6O_{14}S_4\ Na_4$	blue crystals with greenish-bronze luster	NA	1 – 5 at 24.8°C
C. I. Direct Blue 25 $C_{34}H_{22}N_4O_{16}S_4Na_4$	deep purple-blue powder	NA	0.1 – 1 at 21°C
C.I. Direct Red 39 $C_{32}H_{26}N_4O_8S_2Na_2$	red powder	285	< 0.1 at 18°C
C.I. Direct Orange (disodium salt) $C_{28}H_{24}N_6Na_2O_6S$	dark brown powder	NA	< 0.1 at 16°C

Source: Chemfinder (1999)
NA: not available
[a](NTP 1991)

Table 1-3. Physical and chemical properties of DMB

Property	Information	Reference
Molecular weight	212.29	Budavari *et al*. (1996); CRC (1998)
Color	white to reddish crystals or crystalline powder	Budavari *et al*. (1996); CRC (1998)
Physical state	solid crystals or crystalline powder	Budavari *et al*. (1996); CRC (1998)
Melting point (°C)	129 - 131	Budavari *et al*. (1996); CRC (1998)
Boiling point (°C)	200	ACGIH (1986)
Specific gravity	1.0	ACGIH (1986)
Flash point (°C)	85	Budavari *et al*. (1996); CRC (1998)
Solubility: at 19°C		
Water	slightly soluble, < 1 mg/mL	HSDB 1991
95% Ethanol	slightly soluble, < 1 mg/mL	HSDB 1991
Dimethylsulfoxide	soluble, ≥ 100 mg/mL	HSDB 1991
Acetone	soluble, ≥ 100 mg/mL	HSDB 1991
Alcohol	soluble	Budavari *et al*. (1996)
Ether	soluble	Budavari *et al*. (1996)
Dilute acids	soluble	Budavari *et al*. (1996)

DMB is white to reddish crystals or crystalline powder. It is used as an intermediate in the manufacture of dyes and is sensitive to light and prolonged exposure to air (Radian 1991). DMB is a strong oxidizer (NIOSH 1994). When heated to decomposition, it emits toxic fumes of nitrogen oxides (SAX 1984). Its U.S. Environmental Protection Agency (EPA) hazardous waste number is U095, and its RTECS number is NIOSH/DD1225000.

1.3 Identification of metabolites

The metabolism of DMB-based dyes in hamsters, rats, and dogs results in appreciable levels of the free amine, monoacetyl metabolites, diacetyl metabolites, and alkaline hydrolyzable conjugates (AHCs) (see Section 6). Generally, for DMB-based dyes, diacetyl-DMB is the major metabolite, followed by monoacetyl-DMB, and smaller quantities of DMB and AHCs (Lynn *et al.* 1980; Bowman *et al.* 1983). Up to 192 hours after a single oral dose of 12 mg/kg of ^{14}C-labeled C.I. Direct Red 2 to F344/N rats, 20.65% was detected in the urine and 73.51% in the feces (Bowman *et al.* 1982).

2 Human Exposure

2.1 Use

According to the Society of Dyes and Colourists, more than 95 dyes are derived from DMB. Approximately 75% of the DMB produced is used as a dye or as an intermediate in the production of DMB-based dyes. These dyes and pigments are used in printing textiles, as biological stains, and in color photography. Approximately 20% of DMB is used in the production of polyurethane-based high-strength elastomers, coatings, and rigid plastics. DMB also is used as a reagent for detecting gold and chlorine in water and as a curing agent for resins (Budavari 1996; HSDB 1991; Spectrum 1999).

2.2 Production

The United States International Trade Commission (U.S. ITC 1994) reported that DMB was produced by two companies and DMB-based dyes were produced by three companies. Current production volumes for individual producers are not reported because they are confidential for both importers and producers of DMB. Table 2-1 summarizes past total production and import values for those DMB-based dyes for which information was available.

Table 2-1. Production and import values for some DMB-based dyes

Compound	Value (kg)	Year	Source
Tolidines and their derivatives, including DMB (*o*-tolidine) (production)	32,014	1993	U.S. ITC (1994)
DMB dihydrochloride (DMB-2HCl) (imports)	34,200	1984	U.S. ITC (1984)[a]
C.I. Acid Red 114 (production)	172,365	1979	U.S. ITC (1980)[b]
C.I. Acid Red 114 (imports)	9,751	1980	U.S. ITC (1981)[b]
C.I. direct dyes, including Direct Blue 25 (production)	11,228	1993	U.S. ITC (1994)
C.I. direct dyes, including Direct Red dyes 2 and 39, Direct Orange 6, and Direct Blue dyes 14 an 53 (imports)	7,597	1993	U.S. ITC (1994)

[a] Cited by NTP 1991b
[b] Cited by NTP 1991a

2.3 Analysis

The analysis of DMB-derived urinary metabolites is based upon measurement of free diamines through the use of a C_{18} solid sorbent. DMB is eluted, concentrated, injected into a high-performance liquid chromatography system and identified and quantified by monitoring of ultraviolet (UV) absorbance (at 280 or 245 nm) and the electrochemical response. The limit of detection (LOD) for UV analysis is < 2 μg/L, and the limit of quantitation (LOQ) is < 6 μg/L. The LOD for electrochemical detection is < 0.3 μg/L, and the LOQ is < 0.9 μg/L. Recoveries range from 87% to 102% at the 2-μg/L, 10-μg/L, and 20-μg/L levels (Neumeister 1991).

2.4 Environmental occurrence

DMB and DMB-based dyes may be released into the environment as a result of their production and use. Approximately 87% of waste DMB is deposited in water, 5% in terrestrial soil, 5% in aquatic sediments, and 3% in the air (U.S. EPA 1986). In 1996, the most recent year for which information was available, one facility reported releasing a total of 31 lb of DMB, 6 lb into air, and 25 lb into water (U.S. EPA 1996). Another company reported releasing DMB dihydrochloride, but no values were given. None of the DMB-based dyes are documented in the Toxic Release Inventory database, because their releases were not subject to reporting under the Emergency Planning and Community Right to Know Act (U.S. EPA 1996).

2.5 Environmental fate

DMB is found in nature only when it is released into the environment from industrial sources. No information on the environmental fate of DMB-based dyes was found.

2.5.1 Air

DMB has a limited half-life (approximately four hours) in the atmosphere's vapor phase, because it reacts with photochemically activated hydroxyl radicals. No information on DMB photolysis was found, but this process could be important, because DMB absorbs sunlight (HSDB 1991).

2.5.2 Water

DMB is moderately persistent in water, with a half-life between 20 and 200 days (U.S. EPA 1986). DMB released into water covalently binds to humic material in the sediment. Biodegradation of DMB is an important removal process in water, whereas hydrolysis is not. No information on evaporation was found. DMB has a slight tendency to bioconcentrate in aquatic organisms with an estimated bioconcentration factor (BCF) of 35 (a BCF of greater than 1,000 typically results in significant bioaccumulation in aquatic organisms) (HSDB 1991).

2.5.3 Soil

Biodegradation is the most important mechanism by which DMB is inactivated in soil, because DMB does not hydrolyze. It covalently binds to humic material in the soil but has only a moderate tendency to be adsorbed by organic matter. DMB leaching is not rapid in soils. No information about DMB volatilization from soil surfaces was found (Baird *et al*. 1977, cited in HSDB 1991).

2.6 Environmental exposure

Most environmental exposures to DMB occur as a result of contact with industrially contaminated air, water, and soil (HSDB 1991). General population exposure also may occur via contact with paper, fabric, and leather products containing DMB-based dyes.

2.7 Occupational exposure

Most occupational exposures to DMB and DMB-based dyes are of workers in dye manufacturing and processing plants. Occupational exposure may occur through inhalation of dust or mist, through accidental ingestion, or direct contact with the skin. In 1986 and 1987, the U.S. EPA, the American Textile Manufacturers Institute, and the Toxicological Association of the Dyestuffs Manufacturing Industry conducted a joint survey to estimate airborne concentrations of dye dust

in dye weighing rooms of plants where powdered dyes were used to dye and print textiles. The mean airborne concentration of total dye in 24 plants randomly selected for monitoring was estimated to be 0.085 mg/m^3 (U.S. EPA 1990).

The National Institute of Occupational Safety and Health (NIOSH) National Occupational Hazard Survey (NOHS) estimated that 418 workers potentially were exposed to DMB from 1972 to 1974. The National Occupational Exposure Survey (NOES) (NIOSH 1990) reported that 9,639 workers were exposed to DMB between 1981 to 1983. Table 2-2 summarizes the exposure survey data for DMB and DMB-based dyes. NIOSH recommended that exposure to airborne DMB be limited to 0.02 mg/m^3, for any 60-minute work period (NIOSH 1978).

Table 2-2. National estimates of exposure to DMB and selected DMB-based dyes

Compound name	Potentially exposed workers	
	1980s (NOES)	1970s (NOHS)
DMB-based dyes	60,595	16,377
C.I. Direct Red 2	1,450	—
C.I. Direct Red 39	1,450	2,136
C.I. Acid Red 114	13,795	2,852
C.I. Direct Blue 14	813	—
C.I. Direct Blue 25	6,004	1,797
C.I. Direct Blue 53	5,353	1,753
DMB (*o*-tolidine)	9,639	418
DMB-2HCl (*o*-tolidine dihydrochloride)	1,179	—

Source: Provisional data as of January 1, 1990, from the NIOSH National Occupational Exposure Survey (1981 - 1983) and National Occupational Hazard Survey (1972 - 1974), cited in Ruder *et al.* (1990).
—, Not available.

Workers in various other occupations also may be exposed to small quantities of DMB and DMB-based dyes. These workers include water and sewage plant attendants, chemical test tape or kit makers, and swimming pool service representatives. Swimming pool water test kits contain 0.5% to 1.0% DMB, and exposure may occur if they are accidentally emptied into the pool. Chemists also may be exposed in the laboratory when using DMB to detect free chlorine or gold (NTP 1998).

2.8 Biological indices of exposure

The primary biomarker for DMB and DMB-based dyes is urinary DMB. DMB-based dyes are reductively cleaved to DMB in the body. Urine sampling and analysis is performed to complement environmental monitoring in assessment of occupational exposure to these compounds.

2.9 Regulations

The American Conference of Governmental Industrial Hygienists (ACGIH) has classified DMB as a suspected human carcinogen (ACGIH 1991).

U.S. EPA regulates DMB under the Resource Conservation and Recovery Act (RCRA) as a hazardous constituent of waste, under the Clean Air Act (CAA) as a hazardous air pollutant that may be released by certain stationary sources, and under the Toxic Substances Control Act (TSCA), which requires submission of health and safety information. Under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) Trypan Blue has a reportable quantity (RQ) of 10 lb. Under the Superfund Amendments and Reauthorization Act (SARA), DMB hydrochloride, C.I. Trypan Blue, and C.I. Acid Red 114 were placed on a list of toxic chemicals subject to reporting requirements and general threshold quantities for reporting of releases have been established for facilities using or producing these compounds. U.S. EPA regulations applicable to DMB and DMB-based dyes are summarized in Table 2-3.

The Occupational Safety and Health Administration (OSHA) also regulates DMB under the Hazard Communication Standard as a chemical hazard in laboratories (see Table 2-4).

Table 2-3. U.S. EPA Regulations

U.S. EPA Regulations	
Regulatory action	Effect of regulation and other comments
40 CFR 63 – PART 63 – NATIONAL EMISSION STANDARDS FOR HAZARDOUS AIR POLLUTANTS FOR SOURCE CATEGORIES. Promulgated: 57 FR 61992, 12/29/92. U.S. Codes: 7401 et seq.; CAA.	Standards that regulate specific categories of stationary sources that emit (or have potential to emit) one or more hazardous air pollutants are listed in this part pursuant to section 112(b) of the CAA.
40 CFR 63.100ff. – Subpart F – National Emission Standards for Organic Hazardous Air Pollutants From the Synthetic Organic Chemical Manufacturing Industry. Promulgated: 59 FR 19454, 04/22/94.	This subpart applies to chemical manufacturing process units that manufacture DMB and are located at a plant site that is a major source as defined in section 112(a) of CAA. Owners and operators of sources subject to this subpart shall comply with the requirements of subparts G and H of this part.
40 CFR 172 – SUBPART B – Table of Hazardous Materials and Special Provisions. Promulgated: 61 FR 50623, 50624, 09/26/96.	The Hazardous Materials Table in this section designates Trypan Blue as hazardous materials for the purpose of transportation of those materials. The reportable quantity for Trypan Blue is 10 lb (4.54 kg).
40 CFR 192.40 ff. – Subpart E – Standards for Management of Thorium Byproduct Materials Pursuant to Section 84 of the Atomic Energy Act of 1954, as Amended. Promulgated: 48 FR 45947, 10/07/83. U.S. Codes: Sec. 275 of the Atomic Energy Act of 1954, 42 U.S.C. 2022, as added by the Uranium Mill Tailings Radiation Control Act of 1978, Pub. L. 95-604, as amended.	This subpart applies to the management of thorium byproduct materials, such as Trypan Blue, under section 84 of the Atomic Energy Act of 1954, as amended, during and following processing of thorium ores, and to restoration of disposal sites following any use of such sites under section 83(b)(1)(B) of the Act.
40 CFR 261 – PART 261 - IDENTIFICATION AND LISTING OF HAZARDOUS WASTE. Promulgated: 45 FR 33119, 05/19/80. U.S. Codes: 42 U.S.C. 6905, 6912(a), 6921, 6922, 6924(y) and 6938.	This part identifies those solid wastes which are subject to regulation as hazardous wastes under parts 262 through 265, 268, and parts 270, 271, and 124 of this chapter and which are subject to the notification requirements of section 3010 of RCRA. Trypan Blue is given the U.S. EPA Hazardous Waste number U236.
40 CFR 302 – PART 302 – DESIGNATION, REPORTABLE QUANTITIES, AND NOTIFICATION. Promulgated: 50 FR 13474, 04/04/85. U.S. Codes: 42 U.S.C. 9602, 9603, and 9604; 33 U.S.C. 1321 and 1361. Trypan Blue has a statutory final RQ of 10 lb (4.54 kg).	This part designates under section 102(a) of CERCLA 1980 those substances in the statutes referred to in section 101(14) of CERCLA, identifies reportable quantities for these substances, and sets forth the notification requirements for releases of these substances. This part also sets forth reportable quantities for hazardous substances designated under section 311(b)(2)(A) of the CWA.

U.S. EPA Regulations	
Regulatory action	Effect of regulation and 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. Effective date for DMB dihydrochloride is 1/1/95, for Trypan Blue is 1/1/94, and for C.I. Acid Red 114 is 1/1/95.	This part sets forth requirements for the submission of information relating to the release of toxic chemicals under section 313 of Title III of SARA (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, to aid in the development of regulations, guidelines, and standards.
40 CFR 716 – PART 716 – HEALTH AND SAFETY DATA REPORTING. Promulgated: 51 FR 32726, 09/15/86. U.S. Codes: 15 U.S.C. 2607(d). Bisazobiphenyl dyes derived from benzidine and its congeners, *ortho*-tolidine (DMB) and dianisidine (dimethoxybenzidine), have an effective date of 10/04/82 and a sunset date of 10/4/92.	This subpart sets forth requirements for the submission of lists and copies of health and safety studies on chemical substances and mixtures selected for priority consideration for testing rules under section 4(a) of the Toxic Substances Control Act (TSCA) and on other chemical substances and mixtures for which U.S. EPA requires health and safety information in fulfilling the purposes of TSCA.

Source: These regulations in this table have been updated through the 1998 Code of Federal Regulations 40 CFR, July 1, 1998; 21 CFR, April 1, 1998; 29 CFR, July 1, 1998.

Table 2-4. OSHA Regulations

OSHA Regulations	
Regulatory action	Effect of regulation and other comments
29 CFR 1910.1200—Sec. 1910.1200. Hazard Communication. Promulgated 62 FR 42018, 08/04/97.	Requires chemical manufacturers and importers and all employers to assess chemical hazards and to provide information to employees. Hazard Communication program to include labels, material safety data sheets, and worker training.
29 CFR 1910.1450. Promulgated 1/31/90. Amended 58 FR 40191, 7/27/93. OSHA Act: Final rule for occupational exposure to hazardous chemicals in laboratories.	As select carcinogen (IARC Group 2B), dyes that metabolize to DMB are included as chemical hazards in laboratories. Employers required to provide employee information and training and to provide Chemical Hygiene Plan.

Source: These regulations in this table have been updated through the 1998 Code of Federal Regulations 40 CFR, July 1, 1998; 21 CFR, April 1, 1998; 29 CFR, July 1, 1998.

3 Human Cancer Studies

3.1 Background

DMB-based dyes have not been evaluated in human cancer studies as single agents, and most of the epidemiological studies reviewed assessed DMB in chemical mixtures with benzidine derivatives or other arylamines. Benzidine *per se* has been evaluated in a number of epidemiological studies.

3.2 IARC reviews

In IARC (1972), no human carcinogenicity data on DMB were available. In a subsequent IARC evaluation (IARC 1987), DMB was placed in Group 2B (*possibly carcinogenic to humans*). There were, however, no reported human studies. IARC also evaluated C.I. Acid Red 114, a dye that metabolizes to DMB, in 1993. The dye was placed in Group 2B (*possibly carcinogenic to humans*) although the Working Group did not have any human carcinogenicity data (IARC 1993).

Seven arylamines have been classified by IARC. Benzidine-based dyes and 4,4'-methylenebis(2-chloroaniline) (MBOCA) were classified as *probably carcinogenic*, Group 2A, based on a high level of evidence for carcinogenicity in experimental animals. Two industrial chemicals (2-naphthylamine and benzidine), one drug (Chlornaphazine), and two manufacturing processes (manufacture of auramine and magenta) were included in Group 1 on the basis of *sufficient evidence of carcinogenicity in humans*. IARC (1982) concluded that there was sufficient evidence that benzidine is carcinogenic to man. According to IARC (1987), case reports and follow-up studies of workers in many countries had demonstrated that occupational exposure to benzidine is causally associated with an increased risk of bladder cancer; thus benzidine was placed in Group 1 (*carcinogenic to humans*).

3.3 Current studies (see Table 3-1)

In 1996, Ouellet-Hellstrom and Rench (1996) investigated cancer incidence in a cohort of 704 workers employed at a Connecticut chemical plant between 1965 and 1989. The plant produced a variety of chemicals, including arylamines such as dichlorobenzidine, 3,3'-dimethoxybenzidine (DMOB), and DMB. The approximate production volume ratios between 1965 and 1989 were 9:4:1 for dichlorobenzidine, DMOB, and DMB, respectively. Benzidine production stopped before 1965, and only workers never exposed to benzidine at the plant were included in the study. The exposure classification system was developed by a panel of former and current employees based on work processes, potential exposures and job histories, and annual cumulative exposure scores ranging from 0 to 64.4 were calculated for each worker. Cancer cases were identified by three methods: the cohort roster was matched up with cancer cases in the Connecticut Tumor Registry (CTR) through 1990; cancer cases were identified by reviewing death certificates of deceased workers if cancer was a cause or a contributing cause of death; and finally, a mail survey in 1993 was used to determine cancer cases in all members of the cohort who had mailing addresses (potential cases were confirmed by physicians). A total of 27 cancer cases were identified, 23 among male workers and four among female workers. Three of the 23 male cancer cases were non-melanoma skin cancers and were not considered in this study. For men, increased risks were found for cancer of the bladder, with a Standardized Incidence Ratio

(SIR) of 8.3 (95% CI 3.3 - 17.1) and cancer of the testis, with a SIR of 11.4, (95% CI 1.4 - 41.1). For women, breast cancer risk was increased (SIR 1.9, 95% CI 0.4 - 5.6). All bladder cancer cases were potentially exposed to arylamines. Testicular and breast cancer cases were in the non-exposed group. The observed association between bladder cancer and exposure to arylamines increased with increasing exposure (SIRs – 0, 5.5, and 16.4 for no, low, or moderate exposure). All bladder cancer cases were known to be current or former cigarette smokers. Thus, smoking may have contributed to the bladder cancer risk, but probably cannot entirely account for the eight-fold increase in risk (Ouellet-Hellstrom *et al.* 1996).

3.4 Discussion

Arylamines, including benzidine and 2-naphthylamine, have been demonstrated to be human carcinogens. Vineis and Pirastu (1997) reviewed cancer risk in humans resulting from occupational exposure to aromatic amines and tobacco smoking with reference to ecologic, cohort, and case-control studies. Occupational exposures to aromatic amines explain up to 25 percent of bladder cancers. Environmental tobacco smoke as well as occupation may contribute to exposure to aromatic amines. Metabolic polymorphisms, such as the N-acetyltransferase genotype, play a modulating role in the risk of bladder cancer associated with exposure to aromatic amines. The consistent observation of a difference between men and women in bladder cancer risk may indicate gender differences in exposure or in biological determinants of cancer. The study by Ouellet-Hellstrom and Rench (1996) provides additional evidence that arylamine exposure is related to bladder cancer and suggests that DMB exposure may constitute an important component of the exposure. However, since only exposure to total arylamines was evaluated, the study does not directly implicate DMB in cancer risk.

Table 3-1. Cohort studies of workers exposed to DMB

Reference	Population	Exposure	Effects	Potential Confounders
Ouellet-Hellstrom and Rench. (1996) USA. Follow up through 1993	704 workers (585 men and 119 women) first employed at a Connecticut chemical plant between 1965 and 1989. Only workers never exposed to benzidine at the plant were selected. Information on follow-up yielded 8,624 person-years for a follow-up rate of 97% among male employees and 1,660 person-years for a follow-up rate of 97% among women. Expected number based on cancer incidence rates from the State of Connecticut.	Exposure to arylamines established by a committee consisting of four former or current workers knowledgeable about work processes and potential exposures. Scoring system based on intensity of exposure and frequency of contact. Three levels of exposure: none, low, and moderate.	20 Total cancers for males observed. 7 bladder cancers and 2 testicular cancers. **Bladder cancer in men (SIR):** 8.3 (95% CI 3.3 - 17.1). **Bladder cancer in men by exposure level (SIR):** No exposure: 0.0 Low level exposure 5.5 (95% CI 0.7 - 19.8) Moderate level exposure: 16.4 (95% CI 5.3 - 38.2). Smoking and low level exposure 11.6 (95% CI 1.4 - 41.8) Smoking and moderate level exposure: 23.6 (95% CI 7.7 - 55.2). **Testicular cancer in men (SIR):** 11.4 (95% CI 1.4 - 41.1) **Breast cancer in women (SIR):** 1.9 (95% CI 0.4 - 5.6)	All bladder case subjects were known to be current or former cigarette smokers. For other cancers, 37% of male cohort did not indicate smoking status.

4 Studies of Cancer in Experimental Animals

4.1 Carcinogenesis study of C.I. Acid Red 114

The carcinogenic potential of C.I. Acid Red 114, a DMB-based dye, was assessed in male and female Fischer 344/N rats (NTP 1991a). Groups of 50, 35, 65, and 50 male and female rats, 5 weeks old at the time of study initiation, were given drinking water containing C.I. Acid Red 114 at 0, 70 (males only), 150, 300, or 600 (females only) ppm, respectively, for up to 101 weeks. Chemical analysis showed that the dye material was approximately 85% pure and contained 15 organic chemicals closely related to C.I. Acid Red 114 and approximately 5 ppm DMB and < 1 ppm benzidine.

Interim sacrifices of 10 rats per sex were carried out during experimental month 9 (males at 0 and 300 ppm and females at 0 and 600 ppm) and month 15 (males at 1, 10, 150 and 300 ppm and females at 0, 150, 300, and 600 ppm). The number of male rats surviving at termination in the control, 70, 150, and 300 ppm groups were 24/70 (49%), 15/45 (43%), 26/75 (40%), and 1/70 (2%), respectively. The number of female rats surviving at termination in the control, 150, 300, and 600 ppm groups were 36/70 (72%), 13/45 (38%), 6/75 (10%), and 0/70 (0%), respectively. Surviving animals were sacrificed during experimental week 105. Final survival males and females in the high-dose groups was significantly reduced relative to that of concurrent controls ($P < 0.001$). Decreased survival was attributed to sacrifices of moribund rats with treatment-related neoplasms.

Water consumption was monitored to verify palatability of the drinking water solution and to permit determination of daily oral doses of the dye. Water consumption was not affected by the presence of the dye, and estimated daily doses of DMB resulting from the azo reduction of the C.I. Acid Red 114 drinking water doses are summarized in Table 4-1.

Table 4-1. C.I. acid red 114 consumption by male and female F-344/N rats

Experimental week	Drinking water concentration of C.I. Acid Red 114 (ppm)			
	70	150	300	600
	Estimated daily dose of DMB (mg/kg)[a]			
Males				
1 – 13	5.4	11.6	23.0	—
14 – 52	3.9	8.1	16.1	—
53 – 101	3.5	7.6	19.6	—
Females				
1 – 13	—	19.5	35.5	67.1
14 – 52	—	12.8	27.3	47.8
53 – 101	—	9.4	20.5	68.7

Source: NTP (1991a)

—, not tested.

[a] Estimate of DMB molar equivalent doses (mg/kg) based on expected azo reduction of C.I. Acid Red 114 dose.

Chronic oral administration of C.I. Acid Red 114 caused unequivocal dose-related increases in the incidences of benign and malignant tumors of the skin, Zymbal gland, and liver in male and female rats and of the clitoral gland, oral cavity, small and large intestine, and lung in females. Tumor incidences and the results of statistical analyses are summarized in Table 4-2.

Table 4-2. Tumor incidences in F344/N rats administered C.I. Acid Red 114 in drinking water for up to 104 weeks

Tumor type	Tumor incidences/number examined[a]			
	Concentration (ppm)			
	0	70	150	300
Males				
Skin: Basal-cell adenoma or carcinoma	1/50	5/35*	28/65***	32/50***
Sebaceous-cell adenoma or carcinoma	1/50	1/35	5/65	6/50*
Squamous-cell papilloma or carcinoma	1/50	2/35	11/65*	9/50*
Keratoacanthoma	1/50	1/35	4/65	7/50*
Zymbal gland: Adenoma or carcinoma	0/50	0/35	8/65*	7/50*
Liver: Neoplastic nodule or hepatocellular carcinoma	2/50	2/35	15/65*	20/50***
Lung: Alveolar/bronchial adenoma or carcinoma	2/50	2/35	2/65	3/50*
	Concentration (ppm)			
Females	**0**	**150**	**300**	**600**
Skin: Basal-cell adenoma or carcinoma	0/50	4/35*	7/65*	5/50*
Zymbal gland: Adenoma or carcinoma	0/50	3/35	18/65***	19/50***
Clitoral gland: Adenoma or carcinoma	11/48	17/32*	28/62**	23/50*
Liver: Neoplastic nodule or hepatocellular carcinoma	0/50	0/35	19/64***	8/50***
Lung: Alveolar/bronchial adenoma or carcinoma	1/50	2/35	9/65**	4/50
Oral cavity: Squamous-cell papilloma or carcinoma	0/50	3/35	9/65*	6/50*
Small intestine: Polyps or adenocarcinoma	0/50	3/35	1/63	2/50*
Large intestine: Polyps or adenocarcinoma	0/50	1/35	0/64	3/50*

Source: NTP (1991a).
[a]Statistical significance by logistic regression test: $^{*}P < 0.05$; $^{**}P = 0.001$, $^{***}P < 0.001$.

Administration of C.I. Acid Red 114 also was associated with a small but statistically significant increase in the incidence of alveolar/bronchiolar adenomas of the lung in male rats in the high-dose group. Although the incidence of this tumor was low, the researchers noted that administration of the parent amine (DMB) also increased the incidence of pulmonary tumors in male rats (see Section 4.2.4). Based on these tumor incidences, the National Toxicology Program (NTP) concluded that C.I. Acid Red 114 was clearly carcinogenic to male and female F344/N rats under the conditions of this bioassay. Based on these data, the IARC Working Group (IARC 1993) also concluded that there was sufficient evidence to consider C.I. Acid Red 114 to be carcinogenic to experimental animals.

4.2 Carcinogenesis studies of DMB

4.2.1 Oral studies of DMB in rats

Twenty female Sprague-Dawley rats received oral doses of DMB in sesame oil (10 doses at 3-day intervals for a total dose of up to 500 mg/rat). Animals were observed for nine months; of 16 rats that survived nine months, 3 (18%) developed mammary gland carcinomas. Of 132 rats that received only sesame oil, 5 (4%) had a total of 3 mammary gland carcinomas, 1 fibroadenoma, and 5 hyperplasias (Griswold *et al*. 1968, cited in IARC 1972).

4.2.2 Oral studies of DMB in hamsters

DMB was administered to male and female hamsters at dietary concentrations of 0.1% or 0.3%, respectively. These concentrations resulted in total chemical intake of approximately 3.0 and 9.0 g per animal per year, respectively. Animals continued to consume DMB until they died from natural causes (Saffiotti *et al*. 1967, Sellakumar *et al*. 1969, both cited in IARC 1972). Neither DMB dosing regimen increased the incidence of tumors, but dosing of positive control animals with known human and animal carcinogens (including benzidine, dichlorobenzidine, and 2-naphthylamine) increased the incidences of hepatocellular and urinary bladder tumors.

4.2.3 Subcutaneous studies of DMB in rats

DMB suspended in olive oil was administered subcutaneously to Sherman rats (sexes not specified) in weekly doses of 60 mg per animal for a total dose of 5.5 g per animal. No concurrent control groups were used. Five rats developed external auditory canal tumors (most likely Zymbal gland tumors) after day 354 of the study (Spitz *et al*. 1950, cited in IARC 1972).

In another experiment, DMB suspended in sunflower oil was administered to random-bred rats (sex not specified) by subcutaneous injection in weekly doses of 20 mg per rat for 13 months (Pliss and Zabezhinsky 1970, cited in IARC 1972). Of dosed animals that survived for at least eight months (the time of appearance of the initial tumor), 60% (30/50) had 41 tumors with tumors of the Zymbal gland being most frequently observed (66% 20/30). The same investigators implanted pellets of DMB (20 mg in 10 mg of glycerol) into rats. Of 68 animals that survived at least until the appearance of the initial tumor (11 to 12 months), 48 developed 60 tumors, 27 (45%) of which were Zymbal gland carcinomas. Appropriate control groups were not included in these experiments.

4.2.4 Drinking-water studies of DMB in mice

Groups of 120 male and 120 female BALB/c mice were given drinking water containing 0, 5, 9, 18, 35, 70, or 140 ppm of DMB dihydrochloride (Schieferstein *et al*. 1989). Interim sacrifices and histopathological assessments were conducted after 13, 26, 39, 52, 78, or 116 weeks. Water consumption was monitored, and average weekly DMB doses (mg/kg) were determined (Table 4-3).

Table 4-3. Average weekly doses of DMB dihydrochloride administered to BALB/c mice for up to 104 weeks

Drinking water concentration of DMB (ppm)	Average weekly dose of DMB (mg/kg) during the indicated experimental intervals		
	Wk 0 – 4	Wk 48 – 52	Wk 100 – 104
Males			
0	0	0	0
5	3.1	5.0	3.6
9	5.8	9.0	6.4
18	11.3	18.0	13.7
35	22.0	34.6	23.1
70	47.6	70.0	51.8
140	85.4	126.0	95.2
Females			
0	0	0	0
5	4.2	4.2	3.6
9	6.7	6.9	6.8
18	13.7	13.5	11.9
35	27.3	25.9	21.7
70	55.3	52.5	44.1
140	105.0	107.8	109.2

Source: Schieferstein *et al.* (1989)

DMB in oral doses exceeding 100 mg/kg per week was well tolerated by the BALB/c mice of this study, as evidenced by the absence of treatment-related changes in water consumption, body weight gain, or mortality.

Incidences of alveolar-cell adenomas and adenocarcinomas of the lung were increased in a dose-related fashion among males that were either found dead or sacrificed in moribund condition. Similar increases were not observed in females nor in animals randomly selected for interim sacrifice (Table 4-4). The incidences of tumors of the skin, spleen, liver, and Harderian gland were unaffected by the administration of DMB.

Table 4-4. Lung alveolar cell adenomas and adenocarcinomas in BALB/c mice exposed to DMB dihydrochloride in drinking water for up to 104 weeks

Drinking water concentration of DMB (ppm)	Specified sacrifice times (wk)						Animals found dead or moribund
	13	26	39	52	78	112	
	Incidence of lung alveolar cell adenomas and adenocarcinomas/number of animals examined (incidence of adenocarcinomas)						
Males							
0	0/24	0/24	0/8	1/15	11/23	3/10 (1)	5/16 (2)
5	0/24	1/24	0/8	3/16	4/20	5/10 (3)	7/16 (2)
9	0/24	1/24	1/8	1/14	8/18	0/4	5/25 (2)
18	0/24	0/24	0/8	5/14	8/23 (2)	6/10	5/18 (2)
35	0/24	0/24	2/8	2/15	5/18	3/8 (1)	7/24 (6)
70	0/24	0/24	0/8	4/16 (1)	7/21	4/7 (1)	11/20 (5)
140	0/24	0/24	0/8	2/16	8/20	4/7 (1)	13/20 (10)
Females							
0	0/24	0/24	0/8	0/16	4/21	1/7	7/19 (5)
5	0/24	0/24	0/8	1/15	1/23	2/8	4/17 (3)
9	0/24	0/24	1/8	2/16	8/20 (1)	4/9	3/19 (3)
18	1/24	0/24	0/8	1/13	5/21 (1)	4/5 (2)	4/20 (2)
35	0/24	0/24	0/8	3/16	4/20	5/11 (3)	5/17 (2)
70	0/24	1/24	1/8	0/16	2/21	5/10	4/15 (2)
140	0/24	0/24	0/8	4/16 (1)	5/18 (2)	3/11 (1)	4/18 (2)

Source: Schieferstein *et al.* (1989).

4.2.5 Drinking water studies of DMB in rats

Groups of 70, 45, 75, and 70 male and female F344/N rats, five weeks old at the time of study initiation, were given drinking water containing DMB dihydrochloride at 0, 30, 70, or 150 ppm for up to 14 months (NTP 1991b). Although initially planned as a two-year study, this experiment was terminated early because of reduced survival associated with the appearance of treatment-related neoplasms. A scheduled interim sacrifice and histopathological assessment (10 controls and 10 high-dose animals of each sex) was conducted during the ninth month of the study.

Although the incidences of tumors observed in DMB-dosed rats were not significantly elevated at the interim sacrifice, the appearance of any of these neoplasms after only nine months suggested a treatment-associated early onset of some tumors (Table 4-5).

Table 4-5. Incidence of treatment-related tumors in F344/N rats dosed with 0 or 150 ppm of DMB for 9 months

Tumor type	Daily dose (ppm)	
	0	150
	Tumor incidence/number examined	
Males		
Liver: Hepatocellular carcinoma	0/10	2/10
Lung: Alveolar/bronchiolar adenoma or carcinoma	0/10	1/10
Skin: Basal cell carcinoma	0/10	1/10
Preputial gland: Adenoma or carcinoma	0/10	3/10
Small intestine: Mucinous membrane adenocarcinoma	0/10	2/10
Zymbal gland: Carcinoma or adenoma	0/10	3/10
Females		
Lung: Alveolar/bronchiolar adenoma or carcinoma	0/10	1/10
Skin: Squamous cell papilloma	0/10	1/10
Oral cavity: Squamous cell carcinoma (palate)	0/10	1/10
Clitoral gland: Adenoma or carcinoma	0/10	5/10
Zymbal gland: Carcinoma or adenoma	0/10	5/10

Source: NTP (1991b)

Tumor incidences were unequivocally increased in a dose-related manner after 14 months of DMB dihydrochloride administration. Tumor incidences are summarized in Table 4-6. Administration of DMB dihydrochloride increased the incidences of a wide array of malignant and benign tumors in both sexes of F344/N rats. Under the conditions of the experiment, DMB dihydrochloride was clearly carcinogenic to male and female Fischer 344/N rats (NTP 1991b). Further, the array of tumors produced by administration of DMB dihydrochloride was strikingly similar to that produced by administration of C.I. Acid Red 114 (see Table 4-2).

Table 4-6. Tumor incidences in F344/N rats administered DMB hydrochloride in drinking water for 14 months

Tumor type	Daily dose (ppm)			
	0	30	70	150
	Tumor incidences/number examined[a]			
Males				
Skin: Basal cell adenoma or carcinoma	0/60	11/45**	54/75**	30/60**
Sebaceous gland adenoma	0/60	0/45	7/75*	5/60*
Squamous cell papilloma or carcinoma	0/60	2/45	17/75**	27/60**
Keratoacanthoma	1/60	1/45	8/75*	5/60*
Zymbal gland: Adenoma or carcinoma	1/60	3/45	32/75**	36/60**
Preputial gland: Adenoma or carcinoma	2/60	4/45	6/75	9/60*
Liver: Neoplastic nodule or hepatocellular carcinoma	0/60	0/45	35/75**	33/60**
Oral cavity: Squamous cell papilloma or carcinoma	0/60	0/45	4/75	5/60*
Small intestine: Adenomatous polyp or adenocarcinoma	0/60	0/45	4/75	8/60*
Large intestine: Adenomatous polyp or adenocarcinoma	0/60	0/45	6/75*	15/60**
Lung: Neoplasms	1/60	0/45	8/75*	6/60*
Females				
Skin: Basal-cell adenoma or carcinoma	0/60	3/45	10/75**	9/60**
Squamous cell papilloma or carcinoma	0/60	3/45	9/75*	12/60**
Zymbal gland: Adenoma or carcinoma	0/60	6/45*	32/75**	42/60**
Clitoral gland: Adenoma or carcinoma	0/60	14/45**	42/75**	32/59**
Liver: Neoplastic nodule or hepatocellular carcinoma	0/60	0/45	7/74*	4/60*
Oral cavity: Squamous cell papilloma or carcinoma	0/60	3/45	9/75*	13/60**
Small intestine: Adenomatous polyp or adenocarcinoma	0/60	1/45	3/75	5/60*
Large intestine: Adenomatous polyp or adenocarcinoma	0/60	1/45	7/75*	4/60*
Mammary gland: Adenocarcinoma	0/60	1/45	3/75	6/60*

Source: NTP (1991b).
[a] Statistical significance by Fishers exact test: $^{*}P < 0.05$; $^{**}P < 0.001$.

4.3 Transplantability of tumors induced by DMOB, a DMOB-based dye, (C.I. Direct Blue), or a DMB-based dye (C.I. Acid Red 114)

Ulland *et al*. (1989) demonstrated the transplantability of preputial gland and epithelial skin neoplasms induced in Fischer 344 rats during lifetime drinking-water carcinogenesis studies of DMOB, the DMOB-based dye C.I. Direct Blue 15, or the DMB-based dye C.I. Acid Red 114. The neoplasms were retrospectively diagnosed as malignant. Individual neoplasms were not associated with exposure to specific chemicals. Portions of the neoplasms were implanted into the left mammary fat pad of male F344/N rats, and the rate of growth, presence of local invasion and distant metastases, and morphological features were observed following four serial transplants. All transplants were detected early, grew rapidly, and were histomorphologically

similar to the original neoplasms. Metastases were observed with both preputial and skin tumor lines during the serial passages. These results confirmed the malignancy of the preputial gland and skin neoplasms.

4.4 Oncogene activation induced by DMB or C.I. Acid Red 114

A study to detect activation of *ras* oncogenes in tumors induced by DMB or a DMB-based dye explored the possibility that their mechanism of carcinogenesis in rats is the induction of activating point mutations in members of the *ras* gene family (Reynolds *et al.* 1990). Spontaneous tumors and tumors formed in response to the chronic administration of DMB or C.I. Acid Red 114 to rats (as discussed in Sections 4.1 and 4.2) were assayed for the presence of activated oncogenes by the NIH 3T3 DNA mouse fibroblast transfection assay. The results (shown in Table 4-7) confirmed that few activated oncogenes are detected in spontaneous tumors from F344/N rats (1/13 for malignant tumors and 0/25 for benign). In contrast, activated oncogenes were detected in the majority of rat tumors induced by DMB or a DMB-derived dye (5/6 for malignant tumors and 8/10 for benign tumors). The activated oncogene for each tumor is shown in Table 4-8.

Table 4-7. Detection of activated oncogenes in spontaneous tumors and tumors induced by DMB or a DMB-derived dye

Treatment/Tumor type	Frequency (Positive/tested)	Transformation efficiency, foci per μg of DNA	
		Tumor DNA	Transfectant DNA first cycle
Spontaneous[a]			
Benign	0/25		
Malignant	1/13	0.03	1.6
Induced by DMB or C.I. Acid Red 114			
Benign	8/10	0.01 - 0.05	0.03 - 1.05
Malignant	5/6	0.01 - 0.06	0.04 - 0.24

Source: Reynolds *et al.* (1990).
[a] Includes data on 29 spontaneous tumors from F344/N rats reported in an earlier paper.

Table 4-8. Identity and frequency of activated *ras* genes within specific types of induced and spontaneous tumors

Treatment/tumor type	Frequency (positive tumors/tested tumors)	Activated oncogene	
		H-*ras*	N-*ras*
Induced by DMB or C.I. Acid Red 114			
Clitoral carcinoma	4/4	4	—
Basal cell adenoma	4/5	3	1
Basal cell carcinoma	1/1	1	—
Squamous cell carcinoma	3/3	3	—
Trichoepithelioma	1/1	1	—
Fibrosarcoma	0/1	—	—
Mammary fibroadenoma	0/1	—	—
Spontaneous			
Clitoral carcinoma	1/2	1	—
Preputial carcinoma	0/1	—	—
Mammary adenomas	0/11	—	—
Mammary adenocarcinoma	0/2	—	—
Subcutaneous fibromas	0/5	—	—
Lipoma	0/1	—	—
Testicular interstitial cell adenoma	0/5	—	—
Fibrosarcoma	0/2	—	—
Mononuclear cell leukemia	0/3	—	—
Adrenal pheochromocytoma	0/1	—	—
Pancreatic acinar cell adenoma	0/1	—	—
Pancreatic islet cell adenoma	0/1	—	—
Pituitary adenoma	0/1	—	—
Splenic hemangiosarcoma	0/1	—	—
Prostatic adenocarcinoma	0/1	—	—

Source: Reynolds *et al.* (1990).
—, No data reported.

The presence of activated oncogenes in a high percentage of benign tumors induced by DMB or a DMB-based dye suggests that oncogene activation is an early event in the genesis of the tumors. Oligonucleotide hybridization analysis indicated that the H-*ras* oncogenes from the DMB-associated tumors contained mutations at codons 12, 13, or 61. Spontaneously activated H-*ras* genes contained a point mutation at codon 61. The presence of H-*ras* point mutations in DMB-induced benign and malignant neoplasms indicates a probable role for DMB-induced point mutations in the activation of the cellular *ras* genes and in the eventual induction of tumors.

4.4.1 Tumorigenic activity of DMB, DMOB, and dyes based on DMB and DMOB

The pattern of tumors induced by chronic administration of DMB, DMOB, and DMB- and DMOB-based dyes were quite similar (Table 4-9). Such a similar pattern of tumors by the dyes may be taken as evidence of a common mechanism of action for these compounds that would be likely if the dyes were metabolized to the respective amines. In addition, DMB, DMOB, and dyes based on each of these compounds (C.I. Acid Red 114 and C.I. Direct Blue 15, respectively) induced transplantable preputial gland tumors and epithelial gland tumors in F344/N rats (Ulland *et al.* 1989).

Table 4-9. Qualitative tumor responses to DMB, DMB-based dye, DMOB, and DMOB-based dye administered in the drinking water to rats

Tumor type	Amine/Dye[a]			
	DMB	DMB-based C.I. Acid Red 114	DMOB	DMOB-based C.I. Direct Blue 15
Skin				
Basal cell	+	+	+	+
Sebaceous gland	+	+	+	+
Squamous cell	+	+	+	+
Keratoacanthoma	+	+	+	+
Zymbal gland	+	+	+	+
Liver	+	+	+	+
Oral cavity	+	+	+	+
Preputial gland	+	–	+	+
Clitoral gland	+	+	+	+
Mammary gland	+	+	+	+
Small intestine	+	+	+	+
Large intestine	+	+	+	+
Lung	+	+	–	–
Adrenal medulla	–	+	–	–
Brain	+	–	+	+
Mononuclear cell leukemia	+	+	–	+
Mesotheliomas	+	–	+	–

Source: IARC (1993) and NTP (1990, 1991a, 1991b, 1992).

[a]+, Positive tumor response; –, Negative tumor response or not observed.

4.5 Summary

Orally administered DMB is carcinogenic in male and female F344/N rats. Orally administered C.I. Acid Red 114, a DMB-based dye, also is carcinogenic to F344/N rats. The spectrum of tumors induced by C.I. Acid Red 114 was similar to that induced by DMB. The pattern of tumors

induced by chronic administration of DMB, a DMB-based dye, DMOB, and a DMOB-based dye also was similar. Such a similar pattern of tumors is taken as evidence of a common mechanism of action for these compounds, which is likely if the dyes are metabolized to the respective amines. DMB, DMOB, and a dye based on each of these compounds (C.I. Acid Red 114 and C.I. Direct Blue 15, respectively) induce transplantable preputial gland and epithelial gland tumors in Fischer 344/N rats.

5 Genotoxicity

5.1 Prokaryotic Systems

5.1.1 Induction of mutation in Salmonella typhimurium

In tests sponsored by the NTP, DMB dissolved in dimethylsulfoxide was tested at concentrations ranging from 0 to 3333 μg/plate in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98 with and without metabolic activation by S9 liver homogenate derived from rats or hamsters induced with Aroclor 1254. DMB was mutagenic only in *S. typhimurium* strain TA98 in the presence of metabolic activation (Haworth *et al.* 1983).

Similar results were reported in further NTP-sponsored studies by Zeiger *et al.* (1988), who tested DMB dihydrochloride for mutagenicity in *S. typhimurium* using an azo reduction preincubation protocol concentrations ranging from 0 to 666 μg/plate. DMB dihydrochloride was mutagenic only in strain TA98 in the presence of exogenous metabolic activation. It was not mutagenic in strains TA100, TA1535, or TA97 with or without metabolic activation. Elliot and Gregory (1980, cited by IARC 1993) found C.I. Acid Red 114 to be mutagenic in *S. typhimurium* strains TA98, TA100, and TA1538 under reducing conditions.

Another study tested the mutagenic response of *S. typhimurium* strains TA98, TA100, TA1535, and TA1538 (in the presence of S9 metabolic activation) to DMB and its *N*-monoacetyl and *N*, *N*′-diacetyl derivatives. In general, strain TA98 was the most sensitive, followed by TA1538; all three compounds were mutagenic in these strains. In TA100, only the *N*-monoacetylated derivative was mutagenic; DMB produced an equivocal response. None of the compounds was mutagenic in TA1535. The *N*-monoacetylated derivative was more mutagenic than either DMB diamine or the *N*,*N*′-diacetyl derivative in strains TA98 and TA1538 (Reid *et al*. 1984, cited in NTP 1991; Morgan *et al*. 1994).

Urinary metabolites of DMB, obtained from urine extracts from rats orally administered DMB, were more strongly mutagenic in *S. typhimurium* than was DMB itself. Similarly, although the DMB-based dye Evan's blue was not mutagenic in strains TA98 or TA100, its rat urinary metabolites were mutagenic in *S. typhimurium* strain TA98. 3,3′-Dimethyl-*N*-acetylbenzidine and 3,3′-dimethyl-*N*,*N*′-diacetylbenzidine, identified as urinary metabolites of DMB and Evan's blue, were mutagenic in TA98 and TA100 (Tanaka et al. 1982). In another study, DMB also was mutagenic in *S. typhimurium* strains TA98 and TA100 only with S9 metabolic activation (You *et al*. 1993).

Morgan *et al*. (1994) reviewed and summarized the available mutagenicity studies for DMB in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 and DMB-based dyes (C.I. Acid Red 14, C.I. Direct Blue 25, C.I. Direct Red 2, C.I. Direct Red 39) in *S. typhimurium* strains TA98 and TA1538. These data show that DMB and C.I. Acid Red 114 were mutagenic with metabolic activation by S9 derived from the livers of Aroclor-induced male Sprague-Dawley rats and Syrian hamsters. All dyes tested were mutagenic and produced frameshift mutations in *S. typhimurium* strains TA98 and TA1538 using an azo reduction preincubation protocol (preincubation with flavin mononucleotide [FMN] or rat cecal flora).

5.2 Eukaryotic systems

5.2.1 Mutagenicity in Drosophila melanogaster

Valencia *et al.* (1985) tested DMB dihydrochloride for induction of sex-linked recessive lethal mutations in germ cells of adult male *Drosophila melanogaster*. The compound was administered by injection at a concentration of 2,750 ppm in water or in feed at a concentration of 14,000 ppm. The results were positive in the feeding study, but equivocal in the injection study. In a follow-up study, DMB dihydrochloride in feed at 14,000 ppm did not induce reciprocal translocations in the germ cells.

Woodruff *et al.* (1985, cited by IARC 1993) did not observe induction of sex-linked recessive lethal mutations in *D. melanogaster* by the DMB-derived dye C.I. Acid Red 114 administered in feed at 50,000 ppm or by injection at 1,500 ppm.

5.2.2 Mammalian systems in vitro

5.2.2.1 Mouse lymphoma cell mutagenesis assay

In two NTP-sponsored studies (Caspary *et al.* 1988), DMB was mutagenic in the L5178Y mouse lymphoma cell mutagenesis assay both with and without metabolic activation by S9 liver homogenate from Aroclor-induced F344/N rats.

In the first of these studies (Myhr and Caspary 1988), DMB was mutagenic without activation over a narrow range of concentrations just below those that were excessively toxic. Significant increases in mutation frequency (two- to three-fold) were observed at a concentration of 100 μg/mL without activation. The highest concentration that could be tested, 150 μg/mL, induced seven- to eight-fold increases. With activation, the toxicity of DMB was reduced somewhat, and concentrations of up to 200 μg/mL could be tested. However, higher concentrations were required to induce the same mutagenic response, suggesting that the effect of S9 was a deactivation of DMB. At high toxicity, the maximum increases in mutation frequency were about three- to four-fold.

In the second study (Mitchell *et al.* 1988), DMB induced strongly positive, dose dependent increases in mutation frequency both with and without S9 activation. The lowest effective concentration without activation ranged from 26 to 41 μg/mL, and mutation frequencies were increased 3.8- to 7.9-fold at the highest concentrations tested without activation (64 to 80 μg/mL). As observed by Myhr and Caspary (1988), higher concentrations could be achieved with activation and were required to reach mutation frequencies similar to those found without activation.

5.2.2.2 Chromosomal aberrations and sister chromatid exchange

Galloway *et al.* (1987) examined induction of sister chromatid exchanges (SCEs) and chromosomal aberrations in Chinese hamster ovary (CHO) cells by DMB. Results were positive for both end points with and without S9 metabolic activation. SCEs were induced at concentrations ranging from 5 to 50 μg/mL without activation and 500 to 5000 μg/mL with activation. Concentrations effective in inducing chromosomal aberrations ranged from 125 to 180 μg/mL with activation and 225 to 5000 μg/mL without activation

DMB dihydrochloride induced SCEs and chromosomal aberrations in CHO cells in the absence but not in the presence of S9 metabolic activation (NTP 1991b).

5.2.3 Mammalian systems in vivo

5.2.3.1 Micronucleus test

Morita et al. (1997) evaluated the effect of DMB in five separate rodent micronucleus assays carried out by two laboratories. Groups of 4 or 5 male mice, 8 to 10 weeks of age, were treated by intraperitoneal injection once or twice at various dose levels, and bone marrow and/or peripheral blood was analyzed. The highest dose tested ranged from 40 to 60 mg/kg and was based on mortality. No induction of micronuclei was observed in four studies (three with CD-1 mice and one with MS/Ae mice). Marginal, non-dose-related, but statistically significant micronucleus induction was seen in the fifth study (in MS/Ae mice); the evaluators termed this observation "inconclusive."

According to a summary report of the U.S. EPA Gene-Tox Program, DMB induced micronuclei in the bone marrow of hamsters (upon intraperitoneal injection of 25 mg/kg or more) and of rats (upon oral administration of 50 mg/kg or more) (Mavournin *et al*. 1990).

5.3 Summary

DMB and its *N*- and *N,N′*-diacetyl derivatives were mutagenic in *Salmonella typhimurium*, inducing frameshift mutations with metabolic activation by rat or hamster S9 liver homogenate. Urinary metabolites of DMB and DMB-based dyes also were mutagenic in *S. typhimurium*. Preincubation of dyes metabolized to DMB with azo-reducing FMN or rat cecal flora also resulted in mutagenic activity in *S. typhimurium*. DMB but not intact DMB-based dyes induced mutations in *Drosophila melanogaster*. In mammalian *in vitro* systems, DMB was mutagenic to mouse lymphoma cells and induced chromosomal aberrations and SCEs in CHO cells with or without S9 metabolic activation. In mammalian *in vivo* systems, intraperitoneal administration of DMB induced micronuclei in the bone marrow of hamsters, but results for the bone marrow and peripheral blood of mice were equivocal. DMB administered orally induced micronuclei in the bone marrow of rats.

6 Other Relevant Data

6.1 Metabolism of DMB-based dyes

Lynn *et al.* (1980) reported on the metabolism, in rats and dogs, of a series of DMB-based dyes, C.I. Acid Red 114, C.I. Direct Blue 25, C.I. Direct Red 2, and C.I. Direct Red 39 (chemical structures shown in Table 1-1).

Dogs received single oral doses of 100 mg/kg of the dyes, and the urinary excretion of DMB was monitored with gas chromatography (GC) assays. The results of this experiment are summarized in Table 6-1.

Table 6-1. Urinary excretion of DMB by dogs after oral administration of DMB-based dyes (100 mg/kg)

Dye	DMB impurity (ppm)	Dose of DMB as impurity (μg)	DMB excreted in urine during 48 hours after dosing (μg)			Percent of dose[a]
			Experiment 1	Experiment 2	Mean	
C.I. Direct Blue 25	9	13	62	103	82	0.03
C.I. Acid Red 114	< 1	< 1.5	94	175	135	0.04
C.I. Direct Red 2	7	11	BLQ[b]	BLQ[b]	—	—
C.I. Direct Red 39	2	3	BLQ[b]	BLQ[b]	—	—

Source: Lynn *et al.* (1980).
[a] Percent of potential theoretical maximum produced by complete reduction of the azo bonds in the dye.
[b] BLW: Below levels of quantitation, but the presence of DMB was confirmed by GC mass spectrography.

C.I. Direct Blue 25 and C.I. Acid Red 114 were metabolized to DMB, as evidenced by the presence of more of the amine in the urine than could be accounted for by DMB *per se* in the dye sample as an impurity. These results indicate that C.I. Direct Blue 25 and C.I. Acid Red 114 undergo azo reduction to yield the parent amine. A similar conclusion regarding C.I. Direct Red 2 and C.I. Direct Red 39 could not be made, because DMB was consistently below levels of quantitation in the urine of animals dosed with C.I. Direct Red 2 and C.I. Direct Red 39.

In a similar experiment conducted in male Sprague-Dawley rats (Table 6-2), orally administered C.I. Direct Blue 25 (100 mg/kg) was metabolized to DMB in concentrations comparable to those seen in dogs and, in turn, eliminated in the urine. Similar treatment of rats with C.I. Acid Red 114, C.I. Direct Red 2, and C.I. Direct Red 39 did not result in the urinary excretion of quantifiable concentrations of DMB, although the amine was qualitatively identified by GC/mass spectrometry analyses of urine samples. Metabolism of C.I. Direct Blue 25 to DMB in this study also may be inferred because the concentration of DMB in the urine of the dosed animals was 41-fold (< 1 μg as impurity in C.I. Direct Blue 25 and 41 μg in urine), corresponding to approximately 3.5% of the C.I. Direct Blue 25 dose. DMB was detected in the urine of C.I. Acid Red 114-dosed animals below the level at which it was present as an impurity in the dye. DMB was present in the urine of C.I. Direct Red 2- and C.I. Direct Red 39-dosed animals below levels of quantification (Table 6-2).

Table 6-2. Urinary excretion of DMB by rats after oral administration of DMB or DMB-based dyes (100 mg/kg)

Compound	DMB in administered dose (μg)	DMB excreted in urine during 72 hours after dosing (μg)	Percent of dose[a]
DMB	25,290	898 ± 278[b]	3.52 ± 0.99
C.I. Direct Blue 25	< 1	41 ± 3.0	0.06 ± 0.04
C.I. Acid Red 114	< 1	< 0.1	0.01
C.I. Direct Red 2	< 1	BLQ[c]	—
C.I. Direct Red 39	< 1	BLQ[c]	—

Source: Lynn *et al.* (1990).
[a]Percent of potential theoretical maximum produced by complete reduction of the azo bonds in the dye.
[b]Total DMB excreted in 72 hour following single oral dose (mean ± SD of four animals).
[c] BLQ: Below levels of quantification, but the presence of DMB was confirmed by GC/mass spectrometry.

The metabolism of C.I. Direct Red 2, a DMB-based dye, was demonstrated in F344 rats. For this study, the biphenyl portion of the molecule was uniformly labeled. Up to 192 hours after a single oral dose of 12 mg/kg of the ^{14}C-labeled C.I. Direct Red 2 in rats, 20.65 and 73.5% of the radioactivity was detected in the urine and feces, respectively. Sensitive chromatographic analysis, electrochemical gas chromotography (EC/GC), of radioactive compounds revealed that unchanged dye in the feces accounted for only 10.12% of the orally administered dose, indicating extensive cleavage of C.I. Direct Red 2. The urine contained a free amine fraction and in an alkaline hydrolyzable conjugate (AHC) fraction. The free amine fraction consisted of DMB and its mono- and di-acetylated metabolites. The AHC fraction may have contained DMB conjugates because it was hydrolyzed to yield free DMB. After oral administration to rats, ^{14}C-C.I. Direct Red 2 initially appeared at high concentrations in the gastrointestinal tracts (657 μg equivalent DMB in the small intestine and contents) and urinary bladder (2.14 μg equivalent DMB) of rats. It was widely distributed to soft tissues, including adipose tissue, blood, brain, heart, kidney, liver, lung, muscle, spleen, and testes. Tissue concentrations ranged from 0.045 μg equivalent DMB in brain to 0.963 μg equivalent DMB in the liver (two hours post-administration) and (< 0.002 μg equivalent DMB in brain to 3.64 μg equivalent DMB in the liver (72 hours post administration). The urinary concentration was 34.4 μg-equivalent DMB at 72 hours post administration. In comparison, although the parent base of the dye, DMB, is more extensively metabolized, the metabolism of the C.I. Direct Red 2 dye was similar to DMB, yielding the di-acetyldimethylbenzidine as the major product for both dye and DMB (Bowman *et al.* 1982).

In further experimentation, Bowman *et al.* (1983) demonstrated the metabolism of several DMB-based dyes (C.I. Direct Red 39, C.I. Direct Blue 14, C.I. Direct Blue 53, or C.I. Direct Orange 6) in rats. In the study, urinary metabolites in male Fischer 344 rats were monitored following oral administration of 2 mg dose of C.I. Direct Red 39, C.I. Direct Blue 14, C.I. Direct Blue 53, or C.I. Direct Orange 6. Sensitive chromatographic analysis (EC/GC) of metabolites in the urine revealed mainly mono- and di-acetylated DMB, the parent amine (DMB), and alkaline hydrolyzable conjugates in concentrations ranging from 10 μg (for the diacetylated DMB

derived from C.I. Direct Orange 6) to 0.62 μg (for the alkaline hydrolyzable conjugates derived from C.I. Direct Blue 53) at the peak excretion period of 12 to 24 hours post-treatment. At the peak excretion period of 12 to 24 hours post-treatment, total DMB- equivalent of 5.5, 7.9. 5.3, and 14 μg were excreted for C.I. Direct Red 39, C.I. Direct Blue 14, C.I. Direct Blue 53 or C.I. Direct Orange 6 doses, respectively. Excretion of these metabolites was essentially complete within 96 hours.

In similar experiments on the urinary metabolites of C.I. Direct Red 2, a DMB-based dye, Nony *et al.* (1983) demonstrated the metabolism of the DMB-based dye in rats and hamsters. In these studies, male Fischer 344 rats and Syrian golden hamsters were given single oral doses of 100 mg/kg C.I. Direct Red 2 (determined to be sufficiently pure and stable to be used in metabolism studies). Sensitive chromatographic analytical methods, electrochemical gas chromotography (EC/GC), revealed that both species excreted DMB, its mono- and di-acetylated derivatives, and alkaline hydrolyzable conjugates in the urine following ingestion of C.I. Direct Red 2. At the peak excretion period of 48 to 96 hours, a 20 mL volume urine sample of the rats was found to contain 0.088 ppm DMB, 0.299 ppm mono-acetyldimethylbenzidine, 3.98 ppm di-acetyldimethylbenzidine, and 0.293 ppm acid hydrolyzable conjugates. Excretion of these metabolites was essentially complete within 96 hours.

The results of these studies demonstrate the *in vivo* mammalian metabolism of DMB-based dyes to the parent amine. The results also demonstrate that azo-reductive potential varies according to species and dye. Both dogs and rats excreted DMB following administration of C.I. Direct Blue 25, dogs excreted substantial DMB following administration of C.I. Acid Red 114, rats excreted only a trace DMB following administration C.I. Acid Red 114, and neither dogs nor rats excreted quantifiable amounts of DMB following the administration of C.I. Direct Red 2 or C.I. Direct Red 39. In the rat, substantial quantities of the *N*-acetylated metabolites also were formed, but not in the dog. These differences may be due to variability in metabolism of the various dyes, which may lead to differences in formation of parent amine and, consequently, differences in bioactivation and elimination of the carcinogenic metabolites.

6.2 Bacterial metabolism of DMB-based dyes

Cerniglia *et al.* (1982) assessed the abilities of pure cultures of a variety of anaerobic bacteria to reduce the azo linkages in C.I. Direct Red 2, a DMB-based dye. These investigators also studied the ability of bacterial suspensions from the intestinal contents of rats to carry out the reductive cleavage. Both pure cultures of anaerobes and cultures isolated from rat intestinal contents carried out the reductive cleavage. The known organisms varied in the rates at which they reduced C.I. Direct Red 2 (Table 6-5).

Table 6-3. Reduction of C.I. Direct Red 2 by various anaerobic bacteria

Organism	C.I. Direct Red 2 reduction (nmol reduced/mg protein in 8 hours)
Bacteroides thetaitamicron	110.7
Bifidobacterium infantis	110.7
Citrobacter sp.	249.3
Clostridium perfringens	195.0
Lactobacillus acidophilus	233.6
Peptococcus anaerobius	167.1
Clostridium sp.	118.3
Peptostreptococcus productus	78.4
Esherichia coli	2.5

Source: Cerniglia *et al.* (1982).

The bacterial isolate from rat intestine was highly efficient in reducing C.I. Direct Red 2 to DMB. C.I. Direct Red 2 (233 nmol) was added to an incubation medium containing 10^{10} bacterial cells. The mixture was assayed for DMB and C.I. Direct Red 2 at 1, 2, 4, 8, 12, 24, and 48 hours. Production of DMB began promptly and was essentially complete (as evidenced by the absence of C.I. Direct Red 2) within 4 hours.

6.3 Summary

The results of metabolism and elimination studies of DMB-based dyes provide evidence that the dyes are subject to *in vivo* metabolism giving rise to the parent amine. DMB is further metabolized (via *N*-acetylation) and excreted in the urine and feces. Urinary metabolites are primarily the mono- and di-acetylated derivatives of the parent amine. Because the intact dye molecules are not well absorbed from the gastrointestinal tract, the initial metabolic step, azo reduction, most likely takes place in the gastrointestinal tract. Azo reduction of orally administered chemicals can be mediated by the microflora of the intestinal tract, which contains a variety of anaerobic species. Azo reduction, catalyzed by the action of intestinal bacteria, has been demonstrated in the dog, rat, and hamster, as shown by the appearance of DMB and its acetylated metabolites in the urine following oral administration of DMB-based dyes. Further evidence of the role of anaerobic bacteria in reduction of the azo linkages of DMB-based dyes is seen in the fact that this reaction was effectively catalyzed by a number of bacterial species isolated from the gastrointestinal tracts of rats.

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Appendix D: NTP. (1991). Toxicology and Carcinogenesis Studies of 3,3'-Dimethylbenzidine Dihydrochloride in F344/N Rats (Drinking Water Studies). National Toxicology Program Technical Report Series No. 390. pp. D-1 –D-74.

Appendix E: Carcinogen Profile for 3,3′-Dimethylbenzidine (NTP 8th Report on Carcinogens 1998) PP. E-1 – E-3.

3,3′-Dimethylbenzidine
CAS No. 119-93-7

First Listed in the *Third Annual Report on Carcinogens*

Carcinogenicity

There is sufficient evidence for the carcinogenicity of 3,3′-dimethylbenzidine in experimental animals (IARC V.1, 1972). When administered by subcutaneous injection, commercial 3,3′-dimethylbenzidine induced Zymbal gland carcinomas and external auditory canal carcinomas in rats. An IARC Working Group reported that no adequate data were available to evaluate the carcinogenicity of 3,3′-dimethylbenzidine in humans (IARC V.1, 1972; IARC S.7, 1987).

Properties

3,3′-Dimethylbenzidine is a white to reddish crystalline powder that is slightly soluble in water and very soluble in ethanol, ethyl ether, and dilute acids. It is produced as technical-grade dry and paste formulations of various purities. When heated to decomposition, it emits toxic fumes of nitrogen oxides (NO_x).

Use

More than 75% of the 3,3′-dimethylbenzidine consumed is used as a dye or an intermediate in the production of dyestuffs and pigments. According to the Society of Dyers and Colourists, more than 95 dyes are derived from 3,3′-dimethylbenzidine. About 20% of the 3,3′-dimethylbenzidine consumed is used to produce polyurethane-based high-strength elastomers, coatings, and rigid plastics. 3,3′-Dimethylbenzidine is used in small quantities by water companies and swimming pool owners in chlorine test kits, by clinical laboratories in test tapes for the detection of blood, or for the colorimetric determination of chlorine in air or water (IARC V.1, 1972).

Production

The Chem Sources USA directory identified two domestic suppliers of 3,3′-dimethylbenzidine (Chem Sources, 1990). 3,3′-Dimethylbenzidine was imported through the principal United States customs districts in 1989, however, the quantity was unpublished. Imports appear to be the major source of 3,3′-dimethylbenzidine in the United States. In 1986 and 1985, there were three domestic producers of 3,3′-dimethylbenzidine, but no production volumes were reported (USITC, 1987; SRIa, 1986). No producers or production volumes were reported by the USITC in 1984. One producer of 3,3′-dimethylbenzidine hydrochloride was identified in 1983 with no production volume stated (USITC, 1984). The USITC reported imports of 75,000 lb of 3,3′-dimethylbenzidine, and 163,700 lb of its hydrochloride in 1983, compared with the import of more than 5,000 lb of 3,3′-dimethylbenzidine hydrochloride in 1980. Approximately 3.5 million lb of 3,3′-dimethylbenzidine and 240,000 lb of the hydrochloride were imported into the United States in 1979 (USITCa, 1984). The 1979 TSCA Inventory identified one producer of 3,3′-dimethylbenzidine with production volume not specified and four companies importing 115,500 lb in 1977. The CBI Aggregate was between 1 million and 100 million lb (TSCA, 1979). The major company producing 3,3′-dimethylbenzidine in the United States ceased production in 1978; its average annual production was about 200,000 lb.

Exposure

The primary routes of potential human exposure to 3,3′-dimethylbenzidine are inhalation, dermal contact, and ingestion. ACGIH has recommended no threshold-limit value (TLV) time-weighted average (TWA) for 3,3′-dimethylbenzidine because it is regarded as a suspected human carcinogen (ACGIH, 1986). Workers potentially exposed to 3,3′-dimethylbenzidine include dye makers, repackagers of 3,3′-dimethylbenzidine and dimethylbenzidine-based dyes, and personnel in clinical and analytical laboratories. Workers in a variety of occupations may possibly be exposed to small quantities of 3,3′-dimethylbenzidine used for analytical purposes, including water and sewage plant attendants, chemical test tape or kit makers, and swimming pool service representatives. Swimming pool water test kits contain 0.5%-1.0% 3,3′-dimethylbenzidine. Exposure may occur if the test solutions are emptied back into the pool. In 1978, NIOSH estimated that fewer than 100 employees possibly were exposed to large quantities of 3,3′-dimethylbenzidine in the United States, but as many as 200,000 may possibly be exposed to small quantities (NIOSHb, 1979e). The National Occupational Exposure Survey (1981-1983) indicated that 8,676 workers, including 5,383 women, were potentially exposed to 3,3′-dimethylbenzidine (NIOSH, 1984). This estimate was derived from observations of the actual use of the compound (62% of total observations) and the use of tradename products known to contain the compound (38%). The National Occupational Hazard Survey, conducted by NIOSH from 1972 to 1974, estimated that 420 workers were potentially exposed to 3,3′-dimethylbenzidine in the workplace (NIOSH, 1976).

Dimethylbenzidine-based dyes and pigments are metabolized to 3,3′-dimethylbenzidine. Residual levels of 3,3′-dimethylbenzidine may be present in dimethylbenzidine-based dyes and pigments and in the final consumer products. Available data indicate that such contaminants occur in the parts-per-million range. A dimethylbenzidine-based dye was not absorbed dermally to any substantial degree when tested in rabbits.

Regulations

EPA regulates 3,3′-dimethylbenzidine under the Resource Conservation and Recovery Act (RCRA) as a hazardous constituent of waste and has established a reportable quantity (RQ) of 10 lb under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Under the Superfund Amendments and Reauthorization Act (SARA), 3,3′-dimethylbenzidine was placed on a list of toxic chemicals subject to reporting requirements, and general threshold quantities have been established for facilities using or producing the compound. NIOSH recommended a 20 $\mu g/m^3$ ceiling for 3,3′-dimethylbenzidine exposure in the workplace, with no skin contact. OSHA has set standards limiting occupational exposure to 3,3′-dimethylbenzidine. OSHA regulates this compound under the Hazard Communication Standard and as a chemical hazard in laboratories.