FINAL

Report on Carcinogens Background Document for

Dyes Metabolized to 3,3'-Dimethoxybenzidine

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

Prepared for the:
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Contract Number NOI-ES-85421

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'-Dimethoxybenzidine
(3,3'-Dimethoxybenzidine Dye Class)

Carcinogenicity

3,3'-Dimethoxybenzidine-based dyes that are metabolized to 3,3'-dimethoxybenzidine are *reasonably anticipated to be human carcinogens* based on the fact that 3,3'-dimethoxybenzidine is carcinogenic in male and female rats (IARC 1974; NTP 1990, 1998) and that metabolism of 3,3'-dimethoxybenzidine-based dyes to release free 3,3'-dimethoxybenzidine 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'-dimethoxybenzidine-based dye, C.I. Direct Blue 15, is carcinogenic in male and female rats (NTP 1992). Further, the pattern of tumors observed with 3,3'-dimethoxybenzidine (NTP 1990) and C.I. Direct Blue 15 (NTP 1992) is similar to that observed with the structurally similar chemical 3,3′-dimethylbenzidine (NTP 1991a) and the 3,3′-dimethylbenzidine-based dye, C.I. Acid Red 114 (NTP 1991b). 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.

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

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

3,3'-Dimethoxybenzidine is structurally similar to benzidine, a known human carcinogen (IARC 1972, 1982, and 1987; NTP 1998) and 3,3'-dimethylbenzidine, which is reasonably anticipated to be a human carcinogen (NTP 1998). Like benzidine and 3,3'-dimethylbenzidine, 3,3'-dimethoxybenzidine 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'-dimethoxybenzidine-based dyes are chemically equivalent and are readily cleaved by chemical or enzymatic reduction to form free 3,3'-dimethoxybenzidine and the chromophore(s). Reductive cleavage of 3,3'-dimethoxybenzidine and similar dyes 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'-dimethoxybenzidine-based dyes to 3,3'-dimethoxybenzidine also has been shown in studies with rats and dogs (Lynn *et al.* 1980; Bowman *et al.* 1983). By determining the quantities of 3,3'-dimethoxybenzidine and its metabolites excreted following administration of free 3,3'-dimethoxybenzidine versus 3,3'-dimethoxybenzidine-based dyes, Lynn *et al.* (1980) also provided quantitative evidence that each of the two dyes studied was nearly completely metabolized to free 3,3'-dimethoxybenzidine. Metabolism of the dyes to free 3,3'-dimethoxybenzidine in animals is thought to be mediated primarily by bacteria in the gastrointestinal tract (Cerniglia *et al.* 1982; Morgan *et al.* 1994). 3,3'-Dimethoxybenzidine-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'-dimethoxybenzidine, a known bacterial mutagen (Haworth *et al.* 1983).

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

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1 Introduction

Dyes metabolized to 3,3'-dimethoxybenzidine (3,3'-dimethoxybenzidine 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'-dimethoxybenzidine (DMOB) as *reasonably anticipated to be a human carcinogen* and the fact that the azo linkages of DMOB-based dyes are chemically equivalent and are readily cleaved by chemical or enzymatic reduction to form free DMOB 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 are able to absorb and reflect light. Most dyes in use today are synthetic organic compounds.

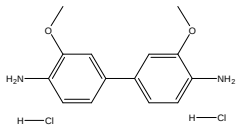
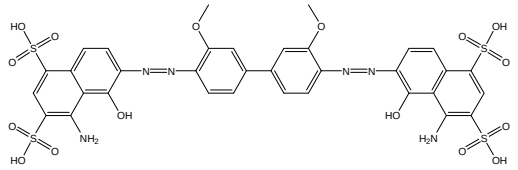
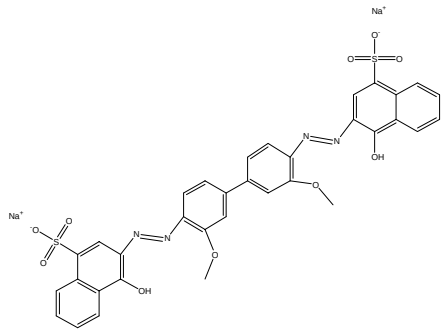
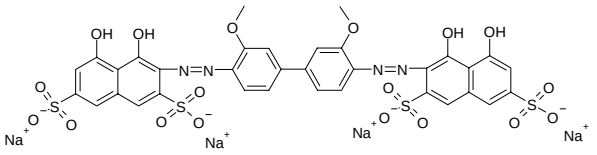
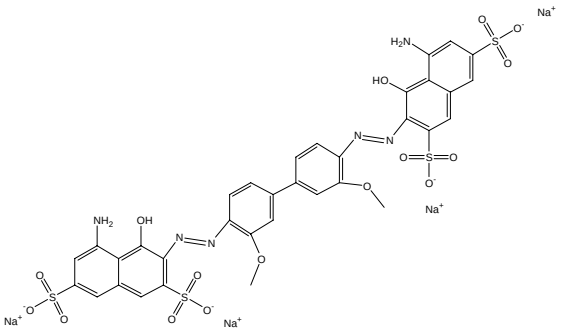
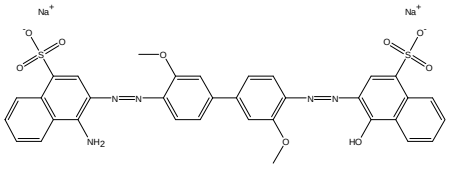
Dyes may be classified according to their chemical structures or their method of application. DMOB-based dyes contain DMOB attached to other substituents by diazo linkages. The dyes evaluated in this report are examples from the class of DMOB-based dyes that have been studied for their potentially carcinogenic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

DMOB ($C_{14}H_{16}N_2O_2$, mol wt 244.29, CASRN 119-90-4) is a methoxylated congener of benzidine and also is known by the following names:

ortho dianisidine
Blue Base
3,3′-dimethoxy-1,1′-biphenyl-4,4′-diamine
4,4′-diamino-3,3′-biphenyldiol dimethyl ether
dianisidine
3,3′-dianisidine
acetamine diazo navy RD
azoene fast blue base
Blue base IRGA B
Blue BN base
Cellitazol B
C.I. azoic diazo component 48
Diacel navy DC
Diazo fast blue B
Fast blue DSC base
Kayaku blue B base
Meisei teryl diazo blue HR
Naphthanil blue B base
Setacyl diazo navy R
Fast Blue
C.I. Disperse Black 6
4,4′-diamino-3,3′-dimethoxybiphenyl
3,3′-dimethoxy-4,4′-diaminobiphenyl
o,o′-dianisidine
acetamine diazo black RD
Amacel developed navy SD
Azogene fast blue B
Blue base NB
Brentamine fast blue B base
Cellitazol BN
Diacelliton fast grey G
Diato blue base B
Fast blue B base
Hiltonil fast blue B base
Lake blue B base
Mitsui blue B base
Neutrosel navy BN
Spectrolene blue B

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

Table 1-1. Examples of DMOB-based dyes

Dye name and formula	CASRN	mol wt	Structure
DMOB-2HCl $C_{14}H_{18}Cl_2N_2O_2$	20325-40-0	317.21	
C.I. Direct Blue 1 C.I. 24410 $C_{34}H_{28}N_6O_{16}S_4$	3841-14-3	904.87	
C.I. Direct Blue 8 C.I. 24140 $C_{34}H_{24}N_4Na_2O_{10}S_2$	2429-71-2	758.68	
C.I. Direct Blue 10 C.I. 24340 $C_{34}H_{24}N_4O_{18}S_4Na_4$	4198-19-0	992.53	
C.I. Direct Blue 15 C.I. 24400 $C_{34}H_{24}N_6O_{16}S_4Na_4$	2429-74-5	992.79	
C.I. Direct Violet 32 C.I. 24150 $C_{34}H_{25}N_5Na_2O_9S_2$	6428-94-0	757.70	
C.I. Direct Black 114	61703-05-7	NA	NA

Source: Chemfinder (1999).
NA: not available.

1.2 Physical-chemical properties

The chemical and physical properties of DMOB are summarized in Table 1-2. DMOB is a colorless, crystalline (sand-like) material that may turn violet upon standing. It is used as an intermediate in making dyes and is sensitive to heat, air, and, prolonged exposure to light (Radian 1991). The U.S. Environmental Protection Agency (EPA) hazardous waste number for DMOB is U091, and its RTECS number is NIOSH/000875000. Table 1-3 summarizes the physical and chemical properties of some DMOB-based dyes (structures are shown in Table 1-1).

Table 1-2. Physical and chemical properties of DMOB

Property	Information	Reference
Molecular weight	244.29	Budavari *et al.* (1996); CRC (1998)
Color	colorless crystals	Budavari *et al.* (1996); CRC (1998)
Physical state	solid crystals	Budavari *et al.* (1996); CRC (1998)
Melting point (^{o}C)	171.5 - 174.5	Budavari *et al.* (1996); CRC (1998)
Boiling point (^{o}C)	NA	Radian (1991)
Vapor pressure (mm Hg at 25°C)	8.8×10^{-9}	HSDB (1991)
Specific gravity	NA	Radian (1991)
Flash point (^{o}C)	206.1	Budavari *et al.* (1996); CRC (1998)
Solubility at 20°C		
Water	slightly soluble, < 0.1 mg/mL	Radian (1991)
95% Ethanol	slightly soluble, < 1 mg/mL	Radian (1991)
Dimethylsulfoxide	soluble, ≥ 100 mg/mL	Radian (1991)
Acetone	soluble, 5 - 10 mg/mL	Radian (1991)
Benzene	soluble	CRC (1998)
Ether	soluble	CRC (1998)
Chloroform	soluble	CRC (1998)

NA: not available.

Table 1-3. Physical and chemical properties of some DMOB-based dyes metabolized to DMOB

Dye name and formula	Color and physical state	Melting point (°C)	Water solubility
DMOB-2HCl $C_{14}H_{18}Cl_2N_2O_2$	off-white powder	268	1 - 5 g/mL at 20°C
C.I. Direct Blue 1 $C_{34}H_{28}N_6O_{16}S_4$	bright greenish-blue solid	NA	0.1 - 0.5 g/100 mL at 23°C
C.I. Direct Blue 8 $C_{34}H_{24}N_4Na_2O_{10}S_2$	bluish-black powder	NA	0.1 - 1 g/100 mL at 18°C
C.I. Direct Blue 10 $C_{34}H_{26}N_4O_{18}S_4Na_4$	blue solid	NA	5 - 10 g/100 mL at 17°C
C.I. Direct Blue 15 $C_{34}H_{24}N_6O_{16}S_4Na_4$	dark blue, microcrystalline powder	> 300	1 - 5 g/100 mL at 20°C
C.I. Direct Violet 32 $C_{34}H_{25}N_5Na_2O_9S_2$	black solid	294 - 300	0.1 - 1 g/100 mL at 17°C
C.I. Direct Black 114	black powder	NA	1 g/100 mL at 21.1°C

Source: Chemfinder (1999) and Budavari *et al.* (1996).
NA: not available.

1.3 Identification of metabolites

The metabolism of DMOB-based dyes to DMOB, in rats and dogs, results in appreciable levels of the free amine, monoacetyl metabolites, diacetyl metabolites, and alkaline hydrolyzable conjugates (AHCs) of metabolites (see Section 6). AHCs account for the major metabolite fraction, followed by appreciable amounts of diacetyl-DMOB and DMOB, with lesser amounts of monoacetyl-DMOB (Bowman *et al.* 1983).

2 Human Exposure

2.1 Use

The major use of DMOB is as an intermediate in the production of DMOB-based dyes used to color leather, paper, plastic, rubber, and textiles. It also is used as a chemical intermediate in the production of DMOB diisocyanate, used in isocyanate-based adhesive systems and as a component of polyurethane elastomers. DMOB also has been used in assays for metals, thiocyanates, and nitriles (NTP 1990; Radian 1991; Spectrum 1999).

2.2 Production

The United States International Trade Commission (U.S. ITC 1994) reported that DMOB was produced by two companies. DMOB-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 DMOB. Table 2-1 summarizes past total production and import values for those DMOB-based dyes for which information is available.

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

Compound	Value (lb)	Year	Source
DMOB (*o*-dianisidine) (imports)	~554,000	1978	U.S. ITC (1980)
DMOB (*o*-dianisidine) (imports)	~106,000	1983	U.S. ITC (1984)
C.I. Direct Blue 15 (production)	270,000	1982	U.S. ITC (1983)
C.I. Direct Blue 15 (imports)	7,716	1980	U.S. ITC (1981)
Direct Blue dyes (including C.I. Direct Blue 15 and 28) (production)	~1280 (581 kg)	1993	U.S. ITC (1994)
Direct Black dyes (including C.I. Direct Black 114) (production)	~16750 (7,597 kg)	1993	U.S. ITC (1994)

2.3 Analysis

Following human exposure to DMOB-based dyes, urinary DMOB can be detected by hydrolysis of urinary metabolites and isolation of the free diamines through the use of a C_{18} solid sorbent. DMOB is 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 0.9 μg/L, and the limit of quantitation (LOQ) is 3.1 μg/L. For electrochemical detection, the LOD is 0.16 μg/L, while the LOQ is 0.70 μg/L. Recoveries range from 87% to 102% at 2-μg/L, 10-μg/L, and 20-μg/L levels (Neumeister 1991).

2.4 Environmental occurrence

DMOB and DMOB-based dyes may be released into the environment as a result of their production and use. Approximately 99% of waste DMOB is deposited in water, 0.5% in terrestrial soil, and 0.5% in aquatic sediments (U.S. EPA 1988). From 1989 to 1996, four companies reported environmental releases of DMOB; no environmental releases of DMOB were reported for 1996. Seven companies reported releasing DMOB dihydrochloride into the environment, but only one had a release above the threshold reportable amount. A chemical

manufacturing division reported a non-point source release of 2 lb and a point source release of 8 lb of DMOB dihydrochloride into the air. None of the DMOB-based dyes had entries in the Toxic Release Inventory database, because their releases were not subject to reporting under the Emergency Planning and Community Right to Know Act (TRI 1996).

2.5 Environmental fate

Because no information is available about the long-term environmental fate of DMOB *per se*, environmental fate estimates are based on analogies with benzidine (HSDB 1991).

2.5.1 Air

Based upon the vapor pressure of DMOB (8.8×10^{-9} mm Hg at 25° C), it should remain almost entirely in the particulate phase in the ambient atmosphere. DMOB has an estimated half-life of two hours in the vapor phase of the atmosphere due, because it reacts with photochemically produced hydroxyl radicals. No information on photolysis is available; however, because DMOB can absorb light at wavelengths greater than 290 nm, this process may play a role in its degradation (HSDB 1991).

C.I. Direct Blue 15 is an example of a DMOB-based dye that is expected to exist in the particulate phase in the ambient atmosphere, because its ionic state is essentially non-volatile. Particulate-phase C.I. Direct Blue 15 may be removed from the atmosphere by wet and dry deposition (HSDB 1996). No other atmospheric fate information was found for any of the other dyes metabolized to DMOB.

2.5.2 Water

DMOB is moderately persistent in water, with a half-life between 20 and 200 days (U.S. EPA 1988). DMOB released into water binds to humic material in the sediment. Biodegradation of DMOB is an important removal process in water, whereas hydrolysis is not. No information on evaporation was found. DMOB has a slight tendency to bioconcentrate in aquatic organisms, with an estimated bioconcentration factor (BCF) of 13.9 (a BCF greater than 1,000 typically results in significant bioaccumulation in aquatic organisms) (HSDB 1991).

For C.I. Direct Blue 15, the major aquatic fate is adsorption to sediment, which increases with decreasing pH. C.I. Direct Blue 15 is expected to be resistant to aerobic biodegradation. Complete anaerobic biodegradation of C.I. Direct Blue 15 by activated sludge inoculum was reported to take seven days. Volatilization of C.I. Direct Blue 15 from water surfaces is not expected to be an important process, as ionic compounds normally do not readily volatilize (HSDB 1996). No aquatic fate information was found for any of the other dyes metabolized to DMOB.

2.5.3 Soil

When DMOB is released into the soil, the amount of adsorption increases with decreasing pH. DMOB also will react with natural substances in the soil, such as clay minerals and Fe(III) as aromatic amines form covalent bonds with humic materials. DMOB was not shown to biodegrade in the MITI test, and only high levels of yeast extracts enhanced biodegradation (HSDB 1991).

C.I. Direct Blue 15 is retained by the ion-exchange process, particularly on clay surfaces, and by adsorption by mineral surfaces such as geothite, which slow or prevent leaching. Because of its ionic nature, C.I. Direct Blue 15 is expected to be resistant to aerobic biodegradation. Complete anaerobic biodegradation of C.I. Direct Blue 15 by activated sludge inoculum was reported to take seven days. Volatilization of C.I. Direct Blue 15 from the soil is not expected to be an important process (HSDB 1996). No terrestrial fate information was found for any of the other dyes metabolized to DMOB.

2.6 Environmental exposure

Most environmental exposures to DMOB and DMOB-based dyes are through contact with contaminated air, water, and soil (HSDB 1991). General population exposure also may occur via contact with paper, fabrics, and leather products containing these dyes and also as a result of consumer use of these dyes.

2.7 Occupational exposure

The primary modes of potential occupational exposure to DMOB and DMOB-based dyes are by inhalation or dermal contact. Most occupational exposures to DMOB occur in dye manufacturing and processing plants during the production of DMOB, during the use and processing of DMOB to make DMOB-based dyes, or during the application of DMOB-based dyes. 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. While DMOB-based dyes were not specifically included in the survey, the results are considered to be representative of DMOB dye dust levels during weighing. The mean airborne concentration of total dye in the 24 plants randomly monitored was estimated to be 0.085 mg/m^3 (U.S. EPA 1990). Current production processes using DMOB and DMOB-dyes, however, generally are closed systems that minimize worker exposure (HSDB 1991). Occupational exposure also may occur in clinical laboratories through use of DMOB as a detecting reagent.

The National Institute of Occupational Safety and Health (NIOSH) National Occupational Hazard Survey (NOHS) estimated that 204 workers potentially were exposed to DMOB from 1972 to 1974. The National Occupational Exposure Survey (NOES) found that 2,482 workers were exposed to DMOB from 1981 to 1983. Table 2-2 summarizes the exposure data for DMOB and DMOB-based dyes. NIOSH has not recommended any occupational exposure limits for DMOB or DMOB-based dyes.

Table 2-2. National estimates of exposure to DMOB and some DMOB-based dyes

Compound	Potentially exposed workers	
	1980s (NOES)	1970s (NOHS)
DMOB-based dyes	99,783	16,166
Pigment Orange 16	42,046	10,858
Pigment Red 41	1,652	100
C.I. Direct Blue 98	21,079	18

Compound	Potentially exposed workers	
	1980s (NOES)	1970s (NOHS)
C.I. Direct Blue 8	1,450	–
C.I. Direct Blue 15	4,528	68
C.I. Direct Blue 1	7,685	1,138
C.I. Direct Blue 80	7,511	1,500
DMOB (*o*-dianisidine)	2,482	120
DMOB-2HCl (*o*-dianisidine, dihydrochloride)	489	–

–: Not studied.
Provisional data as of January 1, 1990, from the NIOSH NOES (1981–1983) and NOHS (1972–1974), cited in Ruder *et al.* (1990).

2.8 Biological indices of exposure

Exposure to DMOB and DMOB-based dyes can be detected in humans via analysis of urinary metabolites of DMOB (see Section 6.1). DMOB-based dyes are reductively cleaved to DMOB, which is further metabolized and excreted in urine and feces as the parent compound and a number of conjugates. Urine sampling and analysis is done to complement environmental monitoring in assessment of occupational exposure to these compounds.

2.9 Regulations

U.S. EPA regulates DMOB under the Resource Conservation and Recovery Act (RCRA) as a hazardous constituent of waste and under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). U.S. EPA also mandates that industrial releases of DMOB and DMOB dihydrochloride be reported by facilities under the Superfund Amendments and Reauthorization Act (SARA). U.S. EPA regulates DMOB and DMOB dihydrochloride under the Toxic Substances Control Act (TSCA), which requires submission of health and safety information. U.S. EPA also regulates C.I. Direct Blue 15 under TSCA. No regulations (U.S. EPA or FDA) were found for other DMOB-based dyes. The applicable U.S. EPA regulations are summarized in Table 2-3.

OSHA regulates DMOB under the Hazard Communication Standard as a chemical hazard in laboratories. OSHA regulations are summarized in Table 2-4. No FDA regulations were found for DMOB.

Table 2-3. U.S. EPA regulations

Regulatory action	Effect of regulation and other comments
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. DMOB is given the U.S. EPA Hazardous Waste number U091.
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 3,3′-DMOB is 1/1/87, for DMOB dihydrochloride 1/1/92, and for C.I. Direct Blue 218 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 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.
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*-toluidine (dimethylbenzidine), dianisidine (DMOB), and C.I. Direct Blue 15 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: The regulations in this table have been updated through the 1998 Code of Federal Regulations 40 CFR, July 1, 1996; 21 CFR, April 1, 1996; 29 CFR, July 1, 1996.

Table 2-4. 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. OSH Act: Final rule for occupational exposure to hazardous chemicals in laboratories.	Dyes metabolized to DMOB classified as carcinogenic (IARC Group 2B) are included as chemical hazards in laboratories. Employers are required to provide employee information and training and to provide a Chemical Hygiene Plan.

Source: The regulations in this table have been updated through the 1998 Code of Federal Regulations 40 CFR, July 1, 1996; 21 CFR, April 1, 1996; 29 CFR, July 1, 1996

3 Human Cancer Studies

3.1 Background

DMOB-based dyes have not been evaluated in human cancer studies as single agents, and most of the epidemiological studies reviewed assessed DMOB 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 DMOB were available. In a subsequent IARC evaluation (IARC 1987), DMOB was placed in Group 2B (*possibly carcinogenic to humans*). The human cancer data, however, was inadequate. IARC also evaluated C.I. Direct Blue 15, a dye that is metabolized to DMOB, in 1993. C.I. Direct Blue 15 was placed in Group 2B (*possibly carcinogenic to humans*); there were no human carcinogenicity data for the dye (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 (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, DMOB, and 3,3'-dimethylbenzidine (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 4 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 can't 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 DMOB exposure may constitute an important component of the exposure. However, since only exposure to total arylamines was evaluated, the study does not directly implicate DMOB in cancer risk.

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

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 cancers for males observed, including 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 cancer 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 studies of DMOB

4.1.1 Oral studies in rats

Several studies of oral administration of DMOB to rats have been reported. In one study, 30 mg of DMOB was administered via gavage in sunflower seed oil to 42 rats, three times a week for three weeks. The dose was reduced to 15 mg after three weeks because of poor survival. The dose of 15 mg was continued for 13 months. Of the18 rats surviving after 14 months, 2 had neoplasms of the Zymbal gland, 1 had a fibroadenoma of the mammary gland, and 1 had an ovarian neoplasm. None of the 50 rats in the control group developed tumors at these sites (Pliss 1963, 1965, cited in IARC 1974 and NTP 1990, 1992).

In another study, male and female Fischer 344 rats (groups of 3 or 14 males and 3 or 15 females) were administered 0.1, 0.3, 1.0, 3.0, 10, or 30 mg of DMOB per rat, five days a week for 52 weeks, followed by a six-month period of observation. A proprietary mixture composed of sodium chloride, sodium carboxymethylcellulose polysorbate 80, and benzyl alcohol in water was used as a vehicle in this study. No controls were reported in this study as cited. Incidences of total neoplasms were increased over those of the 360 pooled vehicle and untreated control rats (no statistical analysis was presented). Tumors were detected at various sites, including the urinary bladder (2 papillomas), mammary gland (3 carcinomas and 2 fibroadenomas), skin (5 carcinomas), intestinal tract (3 carcinomas), and Zymbal gland (8 carcinomas). Tumors appeared as early as day 293, but most were detected upon necropsy, 18 months after the initial DMOB administration (Hadidian *et al*. 1968, cited in IARC 1974 and NTP 1990, 1992).

The carcinogenic potential of DMOB dihydrochloride was evaluated in a drinking-water study in rats of both sexes (NTP 1990). The study was scheduled for a 104-week duration, but was terminated early because of reduced survival of dosed animals attributable to DMOB-associated neoplasms. In this 21-month cancer bioassay, seven-week-old male and female Fischer 344/N rats received drinking water containing DMOB at concentrations of 0, 80, 170, or 330 ppm (corresponding to 0, 6, 12, or 21 mg/kg per day for males and 0, 7, 14, or 23 mg/kg per day for females). The sample sizes for both sexes were 60, 45, 75, and 60 for the control, low-dose, medium-dose, and high-dose groups, respectively. Interim sacrifices of moribund animals or animals bearing large visible tumors were performed throughout the study. The study was terminated at 21 months, when all surviving animals were sacrificed. The tissues and organs of all sacrificed animals were histopathologically examined.

Survival decreased markedly with increasing dosage. Among males, the number of rats surviving at study termination were 44 in the control group and 8 in the low-dose group. None of the male rats in the medium- and-high-dose groups survived the study duration. Among females, 45, 15, and 6 rats survived in the control, low-dose, and medium-dose groups, respectively, and none of the rats in the high-dose group survived.

Tumor incidences and their statistical significance in male and female rats are shown in Tables 4-1 and 4-2. Histopathological examination of the tissues revealed tumors at various sites, including benign and malignant tumors of the skin, Zymbal gland, preputial gland, clitoral gland, mammary gland, uterus, oral cavity, intestine, liver, and mesothelium. An observed increase in the incidence of astrocytomas of the brain may also have been treatment related.

Table 4-1. Tumor incidences in male rats administered DMOB dihydrochloride in drinking water for up to 21 months

	Concentration (ppm) in drinking water			
	0	80	170	330
Tumor type	Tumor Incidence/number examined			
Skin: basal cell or sebaceous gland adenoma or carcinoma	2/60**[a]	33/45**[b]	56/75**[b]	41/60**[b]
Skin: squamous cell papilloma	0/60**[a]	13/45**[b]	28/75**[b]	22/60**[b]
Zymbal gland: adenoma or carcinoma	0/59**[a]	10/45**[b]	25/75**[b]	30/60**[b]
Preputial gland: adenoma or carcinoma	16/60*[a]	12/43	33/73*[b]	29/59*[b]
Oral cavity: papilloma or carcinoma	1/60*[a]	8/45*[b]	10/75*[b]	11/60*[b]
Small intestine: adenocarcinoma	0/60	4/45*[a]	7/75*[a]	5/60*[a]
Large intestine: adenomatous polyp or adenocarcinoma	0/60**[a]	1/45	8/75*[b]	8/60*[b]
Liver: Neoplastic nodule or hepatocellular carcinoma	1/60**[a]	4/45	7/74*[b]	8/60**[b]
Mesothelium: mesothelioma	2/60*[a]	1/45	7/75	6/60
Brain: astrocytoma	0/60	2/44	3/75	1/60

Source: Adapted from NTP (1990).

[a]Statistical significance by Cochran-Armitage Trend Test based on effective rates: *P < 0.05, **P ≤0.001.

[b]Statistical significance by Fisher Exact Test based on effective rates: *P < 0.05, **P ≤0.001.

Table 4-2. Tumor incidence in female rats administered DMOB dihydrochloride for up to 21-months in drinking water

Tumor type	Concentration (ppm) in drinking water			
	0	80	170	330
	Tumor incidence/number examined			
Skin: basal cell adenoma or carcinoma	0/60	4/45*[a]	3/75	2/60
Skin: Squamous cell papilloma	0/60	0/45	3/75	0/60
Liver: Neoplastic nodule or hepatocellular carcinoma	0/60*[b]	1/44	0/75	3/60
Zymbal gland: adenoma or carcinoma	1/60*[b]	12/45**[a]	21/75**[a]	16/60**[a]
Mammary gland: adenocarcinomas	1/60**[b]	2/45	14/75**[a]	20/60**[a]
Oral cavity: papilloma or adenoma	2/60	2/45	6/75	5/60
Large intestine: Adenomatous polyp or adenocarcinoma	0/60*[b]	1/45	1/75	3/60*[b]
Clitoral gland: adenoma or carcinoma	7/58**[b]	27/44**[a]	48/74**[a]	41/45**[a]
Uterus: adenoma or carcinoma	0/60	4/45*[a]	2/75	2/60
Brain: astrocytoma	0/60	1/45	1/75	0/60

Source: NTP (1990).
[a]Statistical significance by Fisher Exact Test based on effective rates: *P < 0.05, **P ≤0.001.
[b]Statistical significance by Cochran-Armitage Trend Test based on effective rates: *P < 0.05, **P ≤0.001.

The incidences of preputial gland and clitoral gland adenomas or carcinomas were significantly increased in the animals administered DMOB hydrochloride. The incidences of preputial and clitoral gland tumors were increased sevenfold and tenfold, respectively, over those in untreated historical controls. Also notable was the earlier appearance of carcinomas in the DMOB-dosed rats (32 weeks in males, 39 weeks in females) than in the controls (87 weeks in males).

DMOB administration also caused significant dose-related increases in the incidences of Zymbal gland adenomas or carcinomas and skin neoplasms. Basal cell or sebaceous gland neoplasms were found in 72% of the DMOB-dosed males, compared with only 3% of the controls. The incidence of these neoplasms was lower in females, but their morphologic type was the same as detected in males; therefore, they were considered to be related to DMOB dihydrochloride exposure. Increased incidences of large and small intestine adenocarcinomas or adenomatous polyps (9% and 5% in high-dose males and females, respectively) also were observed. Large and small intestine adenocarcinomas or adenomatous polyps are rare in rats; none were observed in the 1,601 historical control animals in the National Toxicology Program (NTP) database. The increased incidence of intestinal tumors was considered to be related to DMOB dihydrochloride exposure (NTP 1990).

The NTP concluded that this study provided "clear evidence of carcinogenic activity" of DMOB hydrochloride in male and female Fischer 344/N rats under the conditions of this bioassay (NTP 1990).

4.1.2 Oral studies in hamsters

Groups of 30 male and 30 female Syrian golden hamsters were administered DMOB in the diet at concentrations of 0.1% or 1.0% (1,000 or 10,000 ppm) for 144 weeks. The number of controls was not specified in IARC (1974). The only malignant neoplasm observed was a transitional cell carcinoma of the urinary bladder in one animal after 144 weeks of exposure to DMOB at 1,000 ppm. This neoplasm is rare in hamsters and therefore was attributed to DMOB exposure. Forestomach papillomas were detected in 37% of the high-dose group, compared with 2% of the controls (Saffiotti *et al*. 1967, Sellakumar *et al*. 1969, both cited in IARC 1974).

4.1.3 Drinking water studies in mice

The carcinogenic potential of DMOB dihydrochloride has been evaluated in a drinking-water study in mice of both sexes (Schieferstein *et al*. 1990). In this 112-week cancer bioassay, four-week-old BALB/cStCrlC3Hf/Nctr mice (166 male and 165 female) received drinking water containing DMOB dihydrochloride at 0, 20, 40, 80, 160, 315, or 630 ppm. The animals were scheduled to be sacrificed 13, 26, 39, 52, 78, or 112 weeks after initiation of the bioassay. The high dose level (630 ppm) given over a one-week period is approximately equal to an acute oral bolus dose that would be lethal to half of the animals, assuming that a 30-g mouse drinks 28 g of water per week. Water consumption was depressed in all the dose groups, especially the high-dose group. Although body weight gain was suppressed at the highest dose level during the first year, administration of DMOB dihydrochloride did not affect mortality of either males or females. No increased incidences of neoplasms were observed in any of the tissues examined, which included spleen, Harderian gland, liver, and lung (Schieferstein *et al*. 1990).

4.2 Carcinogenesis study of C.I. Direct Blue 15

The carcinogenicity of C.I. Direct Blue 15, a DMOB-based dye, was evaluated in a 22-month study in 40- to 47- day-old Fischer 344/N rats of both sexes (NTP 1992, IARC 1993). The concentrations of C.I. Direct Blue 15 in distilled drinking water were 0, 630, 1250, or 2500 ppm (corresponding to 0, 45, 90, or 215 mg/kg per day for male rats and 0, 50, 100, or 200 mg/kg per day for female rats). The numbers of male and female rats in the control, low-, medium-, and high-dose groups were 70, 45, 75, and 70, respectively. Ten animals from the control group and the high dose group were sacrificed at the nine-month interim evaluation. Ten additional animals from each group were sacrificed at the 15-month interim evaluation.

The numbers of males surviving the duration of the study were 37 (75%), 8 (24%), 11 (17%), and 2 (4%), respectively, from the 0-, 630-, 1250-, and 2500-ppm dose groups. The numbers of surviving females were 40 (80%), 13 (37%), 22 (35%), and 4 (8%) from the 0-, 630-, 1250-, and 2500-ppm dose groups. Administration of C.I. Direct Blue 15 significantly reduced the survival of both male and female rats. Reduced survival of

dosed animals was attributed to sacrifices of moribund animals necessitated by the appearance of treatment-related neoplasms.

Post-necropsy, histopathological examination of the tissues of the male and female rats revealed benign and malignant neoplasms of the skin, Zymbal gland, preputial gland, clitoral gland, uterus, liver, oral cavity, and small and large intestine. Increased incidences of mononuclear cell leukemia and neoplasms of the brain may also have been treatment-related. Increases in the incidences of Zymbal gland, oral cavity, and intestinal tumors were in the C.I. Direct Blue 15-treated rats were statistically significant and markedly dose-related.

The incidences of squamous cell papillomas or carcinomas of the skin also were significantly increased. A similar increased incidence of basal cell adenomas or carcinomas of the skin was seen in high-dose males (56%, vs. 4% in controls), but not in females. Tumor incidences and their statistical significance are summarized in Table 4-3.

Table 4-3. Tumor incidences in Fischer 344/N rats administered C.I. Direct Blue 15 in drinking water for up to 22 months

Tumor type	Concentration (ppm) in drinking water			
	0	630	1250	2500
	Tumor incidence/number examined[a]			
Males				
Skin: basal cell adenoma or carcinoma	2/50**	9/35**	27/65**	28/50**
Skin: sebaceous gland adenoma	0/50*	1/35	7/65*	3/50*
Skin: squamous cell papilloma or carcinoma	2/50**	4/35	11/65*	19/50**
Zymbal gland: adenoma or carcinoma	1/50**	5/35*	10/65*	20/50**
Preputial gland: adenoma or carcinoma	8/49	5/35	23/64*	9/48
Leukemias	17/50*	19/35*	28/65	20/50*
Liver: neoplastic nodule or hepatocellular carcinoma	0/50**	6/35*	9/65*	11/50**
Oral cavity: squamous cell papilloma or carcinoma	1/50**	10/35**	24/65**	17/50**
Small intestine: adenomatous polyp or adenocarcinoma	0/50	1/35	0/65	2/50
Large intestine: adenomatous polyp or adenocarcinoma	0/50**	1/35	6/65	8/50*
Females				
Skin: basal cell adenoma or carcinoma	1/50	0/35	1/65	0/50

Tumor type	Concentration (ppm) in drinking water			
	0	630	1250	2500
	Tumor incidence/number examined[a]			
Skin: squamous cell papilloma or carcinoma	0/50**	2/35	6/65*	5/50*
Zymbal gland: adenoma or carcinoma	0/50**	4/35	11/65*	17/50**
Clitoral gland: adenoma or carcinoma	7/50**	11/31*	24/64*	27/50**
Leukemias	7/50*	13/35*	27/65**	15/50**
Liver: Neoplastic nodule or hepatocellular carcinoma	0/50**	0/35	2/65	5/50*
Oral cavity: squamous cell papilloma or carcinoma	2/50**	4/35	19/65**	15/50**
Small intestine: adenocarcinoma	0/50*	0/35	1/65	3/50
Large intestine: adenomatous polyp	0/50	0/35	3/65	1/50
Uterus: adenoma or adenocarcinoma	1/50*	0/35	1/65	4/50*

Source: NTP (1992).
[a]Logistic regression tests; this test regards tumors in animals dying prior to terminal kill as nonfatal:
*$P < 0.05$, **$P \leq 0.001$.

Based on the observations in this study, the NTP concluded that there was clear evidence of carcinogenic activity of C.I. Direct Blue 15 in male and female Fischer 344/N rats (NTP 1992). The IARC review of C.I. Direct Blue 15 concluded that it was *possibly carcinogenic to humans* (Group 2B) (IARC 1993).

4.3 Transplantability of preputial tumors induced by DMOB, a DMOB-based dye (C.I. Direct Blue 15), or a DMB-based bye (C.I. Acid Red 114)

Ulland *et al.* (1989) demonstrated the transplantability of preputial gland and epithelial skin neoplasms (epidermal basal cell tumors and epidermal squamous cell carcinomas) induced in Fischer 344/N rats during the lifetime drinking-water studies of DMOB, the DMOB-based dye, C.I. Direct Blue 15, or the DMB-based dye, C.I. Acid Red 114. The neoplasms selected for transformation studies 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 Fischer 344/N rats. 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.

Transplantability also was demonstrated with preputial gland neoplasms induced in Fischer 344/N rats in drinking-water studies of DMOB (NTP 1990) and C.I. Direct Blue

15 (NTP 1992). The transplants were successful, and the transplanted neoplasms appeared to be malignant in nature, growing very rapidly (reaching 3.0 cm in 7 to 9 weeks) with a short latency period. The carcinomas were retrospectively diagnosed, but comparable information was not obtained for preputial gland adenomas. In four serial passages, the transplants did not become less differentiated or anaplastic; however, the transplants maintained their malignant nature despite their well-differentiated morphology. These results confirmed the malignancy of the preputial gland tumors induced by orally administered DMOB and C.I. Direct Blue 15.

4.4 Oncogene activation induced by DMOB or C.I. Direct Blue 15

A study designed to detect activation of *ras* oncogenes in rat tumors induced by DMOB or a DMOB-based dye explored the possibility that their carcinogenic mechanism in rats is the induction of activating point mutations in members of the *ras* gene family (Reynolds *et al*. 1990). Neoplasms obtained from control rats and rats exposed to DMOB or C.I. Direct Blue 15 were assayed for the presence of activated protooncogenes using the NIH 3T3 DNA mouse fibroblast transfection assay (Reynolds *et al*. 1990, NTP 1992). The assay detected activated oncogenes in 21/27 skin, clitoral gland, or preputial gland neoplasms that had been induced by C.I. Direct Blue 15. Activated *ras* oncogenes were detected at a higher frequency (11/13) in tissues of epidermal origin (skin) or histogenetically related tissues (clitoral and preputial gland, 10/14). In comparison, few activated of *ras* oncogenes were detected in spontaneous epithelial neoplasms (1/21) (Reynolds *et al*. 1990). The design and findings of this study are summarized in Tables 4-4 and 4-5.

Table 4-4. Detection of activated oncogenes in tumors occurring spontaneously or induced by DMOB or C.I. Direct Blue 15

Tumor type	Frequency (positive/tested)	Transforming efficiency, foci per µg of DNA	
		Tumor DNA	Transfectant DNA first cycle
Spontaneous[a]			
Benign	0/25	nd	nd
Malignant	1/13	0.03	1.60
Induced by DMOB or C.I. Direct Blue 15			
Benign	1/4	0.01	1.05
Malignant	20/30	0.01 - 0.33	0.20 - 2.00

Source: Reynolds *et al*. (1990)
[a]NIH 3T3 DNA transfection data for 29 spontaneous tumors.
nd: no data.

Table 4-5. Identity and frequency of activated *ras* genes within specific tumor types induced by DMOB or C.I. Direct Blue 15

Tumor type	Frequency (spontaneous tumors)[a]	Activated Oncogene	
		H-*ras*	N-*ras*
Preputial gland adenoma	1/1 (0/1)	1	nd
Preputial gland carcinoma	1/3	1	nd
Clitoral gland carcinoma[b]	8/10 (1/2)	7	1
Basal cell carcinoma	5/6 (np[6])	4	1
Squamous cell carcinoma	6/7 (np)	6	nd
Mammary fibroadenoma	0/2 (0/11)	nd	nd
Mammary adenocarcinoma	0/3 (0/2)	nd	nd
Duodenal adenocarcinoma	0/1 (np)	nd	nd
Subcutaneous. fibroma	0/1 (0/5)	nd	nd

Source: Reynolds *et al.* (1990)
[a] Tumors positive/tumors tested.
[b] One H-*ras* spontaneous activated oncogene observed.
nd: no data; np: not provided.

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

The pattern of the increased tumor incidences observed with C.I. Direct Blue 15 was similar to that seen with DMOB and 3,3′-dimethylbenzidine (DMB), a structural analog of DMOB. Similar exposure to C.I. Acid Red 114, a dye based on DMB, also resulted in a similar pattern of tumors. A comparison of the tumorigenic response in rats for DMOB and DMOB-based dyes with DMB and DMB-based dyes is presented in Table 4-6.

In addition, DMOB, DMB and dyes based on each of these compounds (C.I. Direct Blue 15 and C.I. Acid Red 114, respectively) induced transplantable preputial gland and epithelial gland tumors in F344/N rats (Ulland *et al*. 1989). Activated *ras* oncogenes were also detected in DMOB- and C.I. Direct Blue 15-induced tumors at higher frequencies than those that arose spontaneously (Reynolds *et al*. 1990).

Table 4-6. Qualitative tumor responses of rats administered DMOB, a DMOB-based dye, DMB, or a DMB-based dye in drinking water

Tumor type	Amine/Dye[a]			
	DMOB	DMOB-based C.I. Direct Blue 15	DMB	DMB-based C.I. Acid Red 114
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.

4.6 Summary

Oral administration of DMOB is carcinogenic in rats and hamsters. The pattern of DMOB-induced tumors is similar to that seen with C.I. Direct Blue 15, a dye metabolized to DMOB. The one study of DMOB in mice failed to detect an increased incidence of tumors. The pattern of tumors induced by chronic administration of DMOB, a DMOB-based dye, DMB, and a DMB-based dye is strikingly 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. DMOB, DMB, and a dye based on each of these compounds (C.I. Direct Blue 15 and C.I. Acid Red 114, respectively) induce transplantable preputial gland and epithelial gland tumors in Fischer 344/N rats. In addition, activated *ras* oncogenes are detected in tumors induced by DMOB or C.I. Direct Blue 15 at higher frequencies than in spontaneously occurring tumors.

5 Genotoxicity

5.1 Prokaryotic systems

5.1.1 *Induction of mutation in* Salmonella typhimurium

DMOB has been extensively studied for the induction of gene mutations in *Salmonella typhimurium.* In tests sponsored by the NTP (1992), DMOB dissolved in dimethylsulfoxide (DMSO) was tested at multiple concentrations (0 to 10,000 μg/plate) in three different laboratories, in various *S. typhimurium* strains with and without metabolic activation by S9 liver homogenate from Aroclor-induced rats and hamsters. Overall, DMOB was mutagenic with exogenous metabolic activation in strains TA98 and TA100 (Haworth *et al.* 1983; NTP 1992). One laboratory reported a significant response in strain TA98 without metabolic activation, and another laboratory observed a weakly positive response in strain TA1535 in the presence of hamster S9 liver homogenate.

Another study tested the mutagenic response of various *S. typhimurium* strains (with and without S9 liver homogenate from Aroclor-induced rats or hamsters) to DMOB and the corresponding *N*-monoacetyl and *N,N′*-diacetyl derivatives. In general, TA98 was the most sensitive strain, followed by TA1538; all three compounds were mutagenic in these strains. DMOB was more mutagenic in the presence of S9 liver homogenate from rats than with S9 from hamsters. The *N*-monoacetylated derivative was more mutagenic than DMOB or the *N,N′*-diacetyl derivative (Reid *et al*. 1984, cited in Morgan *et al*. 1994). The mutagenic activity of DMOB and DMOB dihydrochloride was tested in *S. typhimurium* strains TA100 and TA98 with and without rat S9 metabolic activation, at concentrations ranging from 0 to 5 μg/mol. Both the free base and dihydrochloride salt forms of DMOB were mutagenic with metabolic activation, and no appreciable difference in mutagenic activity between the two forms was observed (Messerly *et al*. 1987).

In another gene mutation assay, DMOB at concentrations of 5, 50, or 100 μg/plate was mutagenic in *S. typhimurium* strains TA98 and TA1538 with S9 metabolic activation (Sariaslani and Stahl 1990). Other studies have confirmed that DMOB is mutagenic in strain TA98 with metabolic activation and reported a weak mutagenic response in strain TA100 with activation (You *et al*. 1993).

A number of dyes metabolized to DMOB (C.I. Direct Blue 1, C.I. Direct Blue 8, C.I. Direct Blue 10, C.I. Direct Blue 15, and C.I. Direct Violet 32) are not mutagenic in the absence of conditions that result in the reduction by azo bonds. However, all DMOB-based dyes tested in an azo-reductive system (rat cecal flora mix or flavin mononucleotide [FMN]) were found mutagenic in *S. typhimurium* strains TA98 or TA1538 (Morgan *et al.* 1994). The results of this study are summarized in Table 5-1.

Table 5-1. Mutagenicity of DMOB and dyes metabolized to DMOB in *S. typhimurium*

Compound	% Purity	Metabolic activation			
		None	S9	FMN	Cecal
DMOB	98	-	+	+	+
C.I. Direct Blue 1	-	-	-	+	NT
C.I. Direct Blue 8	30	-	-	+	+
C.I. Direct Blue 10	48	-	-	+	+
C.I. Direct Blue 15	50	-	-	+	+
C.I. Direct Violet 32	-	-	-	+	+

Source: Morgan *et al.* (1994).
S9: standard (aerobic) preincubation test procedure
FMN: FMN-supplemented S9 for reductive metabolism
Cecal: rat cecal flora suspension for anaerobic metabolism
(-): not mutagenic
(+): mutagenic
NT: not tested

C.I. Direct Blue 15, a dye that is metabolized to DMOB, yields DMOB upon metabolic reduction of the azo bonds and is thus considered potentially genotoxic (Ashby and Tennant 1988; Mortelmans *et al*. 1986, both cited in NTP 1992). In the NTP study (NTP 1992), C.I. Direct Blue 15 was not mutagenic in various *S. typhimurium* strains (with or without rat or hamster liver S9 metabolic activation) at various concentrations (100 to 10,000 µg/plate). However, when tested after reductive (rat cecal bacteria or FMN) metabolism, it was mutagenic in *S. typhimurium* strain TA1538 with S9 activation at concentrations of 0.25, 0.50, or 1.00 µM (NTP 1992). Other studies have confirmed that C.I. Direct Blue 15 is mutagenic in *S. typhimurium* strains TA98, TA100, and TA1538 when reductive metabolism precedes incubation (Gregory *et al*. 1981; Brown and Dietrich 1983; Prival *et al*. 1984; Reid *et al*. 1984, all cited in NTP 1992).

C.I. Direct Blue 15 was found to be mutagenic in *S. typhimurium* strain TA98 and the arabinose-resistant tester strain SV50 in the presence of hamster or rat liver S9 metabolic activation. The concentrations tested ranged from 0.10 to 3.00 mg/plate. SV50 was less sensitive than TA98 in detecting mutagenic response (Krishna *et al*. 1986). In this study, DMOB was non-mutagenic in the arabinose-resistant tester strain SV50 and mutagenic in *S. typhimurium* strain TA98.

5.1.2 *Mutagenicity in* Escherichia coli

DMOB failed to induce DNA damage in *Escherichia coli* in the absence of S9 metabolic activation (Fluck *et al.* 1976, cited in NTP 1990).

5.2 Eukaryotic systems

5.2.1 *Mutagenicity in* Drosophila melanogaster

DMOB did not induce sex-linked recessive lethal mutations in adult male *Drosophila melanogaster* administered DMOB in feed at 100 ppm or by injection at 200 ppm (Yoon *et al*. 1985, cited in NTP 1990).

5.3 Mammalian systems

5.3.1 In vitro assays

5.3.1.1 Mouse lymphoma cell mutagenesis assay

In two NTP-sponsored studies (Caspary *et al.* 1988), DMOB dihydrochloride was mutagentic in the L5178Y mouse lymphoma cell mutagenesis assay both with and without metabolic activation by S9 liver homogenate from Fischer 344 rats.

In the first study (Myhr and Caspary 1988), the assays were conducted with no exogenous activation and with activation by S9 liver homogenate from non-induced Fischer 344/N rats. DMOB was mutagenic under non-activation conditions over a narrow concentration range (60 to 90 μg/mL) just below the toxic concentration (≥ 90 μg/mL). Addition of S9 caused a reduction in toxicity. A two-fold increase in mutation frequency was observed at 75 μg/mL. At 250 μg/mL (which exceeded the apparent solubility limit), the mutation frequency increase ranged from 1.6- to 2.2-fold. Insoluble doses of 300 μg/mL induced a three-fold increase in mutation frequency. The primary effect of addition of S9 was to reduce the toxicity of DMOB dihydrochloride without changing the magnitude of the mutagenic activity at toxic doses (Myhr and Caspary 1988).

In the second study (Mitchell *et al.* 1988), the mouse lymphoma assay was conducted with and without S9 liver homogenate from Aroclor-induced Fischer 344 rats. DMOB dihydrochloride was mutagenic in the absence of S9, inducing a three- to four-fold mutation frequency increase at concentrations of 64 μg/mL and 100 μg/mL. In the presence of S9, the toxicity was reduced, and concentration ranges were approximately five times as high. A twofold increase in mutation frequency was observed at concentrations of 328 μg/mL and 437 μg/mL.

5.3.1.2 Chromosomal aberrations (CA)

DMOB dihydrochloride induces CA in Chinese hamster ovary (CHO) cells with and without metabolic activation (Galloway *et al*. 1987, cited in NTP 1990). When first reported, the results of the CA tests were considered negative, but later statistical reanalysis of the data indicated that the results were weakly positive without S9 metabolic activation and positive with S9 activation (Galloway *et al*. 1985, 1987, cited in NTP 1990). Concentrations ranged from 0.005 to 5000 μg/mL.

The DMOB-based dye C.I. Direct Blue 15 failed to induce CA in CHO cells. Concentration ranges for this assay were 1500 to 2250 μg/mL without metabolic activation and 2000 to 2500 μg/mL with metabolic activation. Reductive metabolism was not used in the chromosomal aberrations assay (NTP 1992).

5.3.1.3 Sister chromatid exchanges

DMOB dihydrochloride induced sister chromatid exchanges (SCEs) in CHO cells both with and without S9 metabolic activation. Concentrations ranged from 0.005 to 5000 μg/mL. In one of the two laboratories conducting these cytogenetic tests, the weakly positive SCE result without S9 activation occurred under delayed harvest (3 to 5 hours), because DMOB dihydrochloride induced a delay in the cell division cycle. The positive

results obtained by the other laboratory occurred at lower concentrations of DMOB dihydrochloride that did not affect cell cycle time (Galloway *et al*. 1985, cited in NTP 1990).

C.I. Direct Blue 15 did not induce SCEs in CHO cells. Concentrations ranged from 250 to 750 μg/mL without metabolic activation and 83.3 to 2500 μg/mL with metabolic activation. A 20% increase in SCEs per chromosome of culture would have been classified as a positive result. Reductive metabolism was not used in this assay (NTP 1992).

5.3.2 In vivo *assays*

5.3.2.1 Chromosomal aberrations

A single 100-mg/kg dose of DMOB was injected into four mice. DMSO (2 mL/kg) was used as a negative control, and 7,12-dimethylbenz[*a*]anthracene (DMBA) (100 mg/kg) was a positive control. DMOB caused a statistically significant increase in CA in the bone marrow of mice relative to DMSO controls (aberrant cells = 5.25 $\pm$ 0.96%, mitotic indices = 2.05 $\pm$ 0.15%). DMOB was not, however, nearly as genotoxic as the positive control, DMBA (You *et al.* 1993).

5.4 Summary

DMOB is mutagenic in *Salmonella typhimurium* with exogenous metabolic activation. C.I. Direct Blue 15, a DMOB-based dye, also is mutagenic in *S. typhimurium* with metabolic activation and in the presence of azo-reductive systems that form DMOB. DMOB induces mutations in the mouse lymphoma cell assay and causes CA and SCE in CHO cells, in the presence or absence of exogenous metabolic activation. DMOB also induces chromosomal aberrations *in vivo* in the bone marrow of mice.

6 Other Relevant Data

6.1 Mammalian absorption, distribution, metabolism, and excretion of DMOB and DMOB-based dyes

In a study of DMOB-based dye absorption, metabolism, and excretion, five female mongrel dogs received single oral doses of 100 mg/kg of C.I. Direct Blue 15 or C.I. Direct Blue 1. Free DMOB, as an impurity in each dye sample, was determined. The urinary excretion of free DMOB also was monitored using gas chromatography. C.I. Direct Blue 15 and C.I. Direct Blue 1 were metabolized to DMOB as indicated by the presence of more amine in the urine than could be accounted for by the presence of free DMOB as an impurity in the dye sample. This is an indication that C.I. Direct Blue 15 and C.I. Direct Blue 1 undergo azo reduction to yield the parent amine, DMOB. There was substantial variation in the percentage of the dose of the dyes excreted as free DMOB. The results of this study are summarized in Table 6-1 (Lynn *et al*. 1980).

Table 6-1. Urinary excretion of DMOB by dogs after oral administration of DMOB-based dyes

Dye	DMOB impurity (ppm)	Dose of DMOB as impurity (μg)	DMOB excreted in urine during 48 hours after dosing (μg)			Percent of dose[a]
			Experiment 1	Experiment 2	Mean	
C.I. Direct Blue 15	46	69	61	168	114	0.03
C.I. Direct Blue 1	18	27	441	119	280	0.08

Source: Lynn *et al.* (1980)
[a]Percent of potential theoretical maximum produced by complete reduction of the azo bonds in the dye.

In a similar study, C.I. Direct Blue 15 and C.I. Direct Blue 1 were administered by gavage (100 mg/kg) to male Sprague-Dawley rats to study the absorption, metabolism, and excretion of the dyes in rats (Lynn *et al.* 1980). The free DMOB impurity in the C.I. Direct Blue 15 and C.I. Direct Blue 1 samples were measured as 1 and <1 μg, respectively. DMOB was excreted in the urine of the rats at concentrations of 13.0 ± 12.9 and 42.5 ± 27.7 μg, respectively, 72 hours after administration of C.I. Direct Blue 15 and C.I. Direct Blue 1. These urinary concentration levels of free DMOB correspond to 0.17 ± 0.18 and 0.55 ± 0.37 percent, respectively, of the potential theoretical maximum produced by complete reduction of the azo bonds of the administered C.I. Direct Blue 15 and C.I. Direct Blue 1 doses. In comparison, the free DMOB was eliminated in the urine of rats in higher concentrations than in the urine of dogs from the same doses of C.I. Direct Blue 15 and C.I. Direct Blue 1. More detailed results from these urinary excretion studies of benzidine-, DMB-, and DMOB-based dyes in rats and dogs are presented in Appendix C (Tables C-1 and C-2).

DMOB-based dyes, including C.I. Direct Blue 15, have been reported to be metabolized to DMOB in humans as indicated by the detection of DMOB in the urine of three of 22 workers who dried and ground two DMOB-based dyes (NIOSH 1981; Rodgers *et al.* 1983, cited in NTP 1992). Azo dyes containing DMOB can also be reduced by rat liver azoreductases to DMOB (Martin and Kennelly 1981, cited in NTP 1992).

Bowman *et al.* (1982) demonstrated the metabolism of C.I. Direct Blue 15, a DMOB-based dye, in male Fischer 344 rats. In this study, the biphenyl portion of the molecule was uniformly labeled.. Approximately 18.8% of the [^{14}C] administered was recovered in the urine of the rats given C.I. Direct Blue 15 (12 mg dye/kg body weight or molar equivalent DMOB by oral gavage). Intact dye in the feces accounted for 12% of the orally administered dose, with 84% of the fecal [^{14}C] resulting from unidentified metabolic products. In comparison, when rats were administered ^{14}C-labeled DMOB, 35% and 74.4% of the [^{14}C] was recovered in the urine and feces, respectively. The excretion of [^{14}C] in the feces and urine peaked at 8 to 16 hours after dosage, although detectable amounts of [^{14}C] were still being excreted 144 to 192 hours after the single oral dose of 12 mg/kg of the ^{14}C-labeled dye . Analysis of urinary metabolites after oral administration of C.I. Direct Blue 15 revealed that radioactivity was excreted in a free amine fraction and in an alkaline hydrolyzable conjugate (AHC) fraction. The free amine fraction was comprised of DMOB (0.22% of the dose), its monoacetylated metabolite (0.27%), and its diacetylated metabolite (0.22%). The AHC fraction contained DMOB (0.48% of the dose). DMOB is more extensively metabolized and excreted than the dye. Diacetylated DMOB was the major metabolite observed following administration of DMOB-based dyes. Following administration of DMOB, most of the dose found in urine was in the AHC fraction (1.56%). Other compounds found in urine were DMOB (1.18%), monoacetylated-DMOB (0.35%), and diacetylated-DMOB (0.93%).

After oral administration of ^{14}C-labeled C.I. Direct Blue 15 to rats, [^{14}C] concentration was initially high in the gastrointestinal tract, with subsequent time-related, widespread distribution of radioactivity to soft tissues.

In further experimentation, Bowman *et al.* (1983) demonstrated the metabolism of several DMOB-based dyes (C.I. Direct Blue 8, C.I. Direct Blue 10, C.I. Direct Violet 32, or C.I. Direct Black 114). In this study, urinary excretion of DMOB and its metabolites was observed in the urine of male Fischer 344 rats up to 96 hours after the oral administration of a single dose of 2 mg of C.I. Direct Blue 8, C.I. Direct Blue 10, C.I. Direct Violet 32, or C.I. Direct Black 114. Sensitive chromatographic analysis (EC/GC) of metabolites in the urine revealed mainly mono- and di-acetylated-DMOB, the parent amine (DMOB), and alkaline hydrolyzable conjugates in concentrations ranging from 15 μg (for alkaline hydrolyzable conjugates derived from C.I. Direct Violet 32) to 0.07 μg (for mono-acetylated DMOB derived from C.I. Direct Blue 8) at the peak excretion period of 12 to 24 hours post-treatment. At the peak excretion period of 12 to 24 hours post-treatment, a total DMOB μg-equivalent of 4.9, 12, 11, and 27 were excreted for C.I. Direct Blue 8, C.I. Direct Black 114, C.I. Direct Blue 10, C.I. Direct Violet 32 doses, respectively. Excretion was essentially complete within 96 hours.

Rodgers *et al.* (1983, cited in NTP 1990) investigated the metabolism of ^{14}C-labeled DMOB administered intravenously to male Fischer 344 rats. Thirty minutes after dosing,

less than 2% of unchanged ^{14}C-labeled DMOB was recovered. Within 72 hours, 70% of the total ^{14}C-labeled dye administered was excreted in the bile. In intact rats, 50% of the dose was found in the intestinal tract after 2 hours. After either oral or intravenous administration, 50% of the dose was excreted in feces and 30 to 40% in urine within three days. Of the [^{14}C] remaining in the animal, 45% was present in the liver in the form of covalently bound metabolites; more than 90% of the urinary radiolabel was in the form of metabolites. Unmetabolized DMOB accounted for 3 to 9% of the dose, and less than 5% was associated with acetyl-DMOB. The proposed metabolic pathway is shown in Figure 6-1.

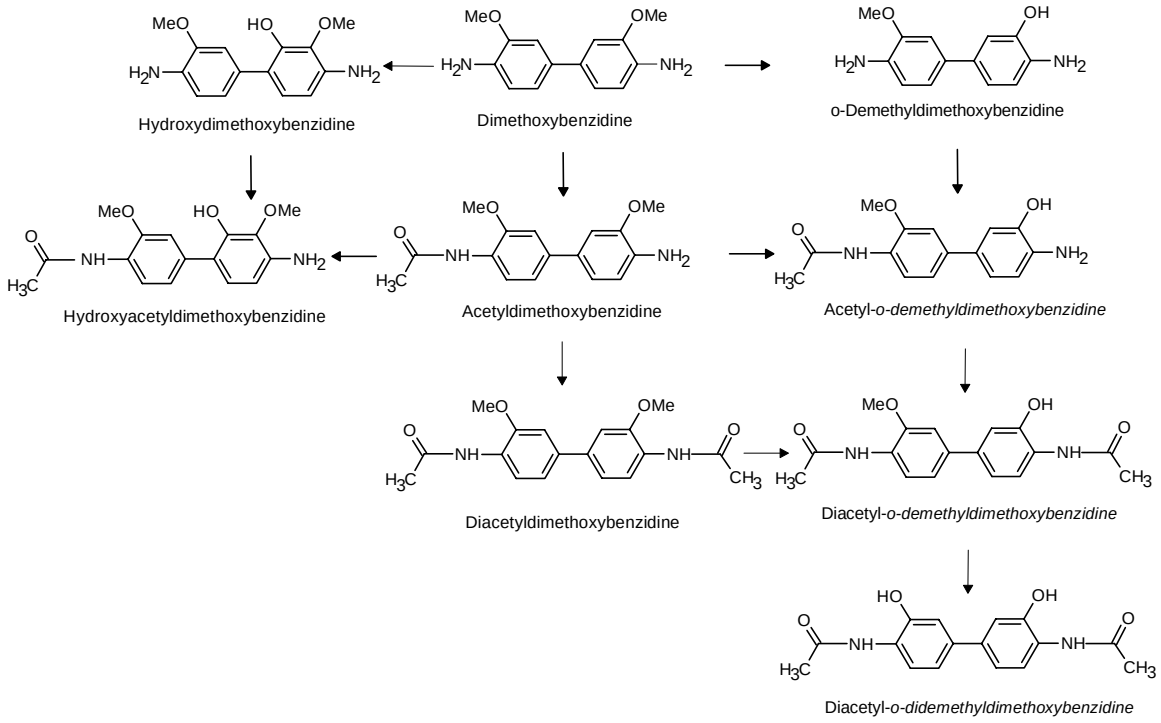
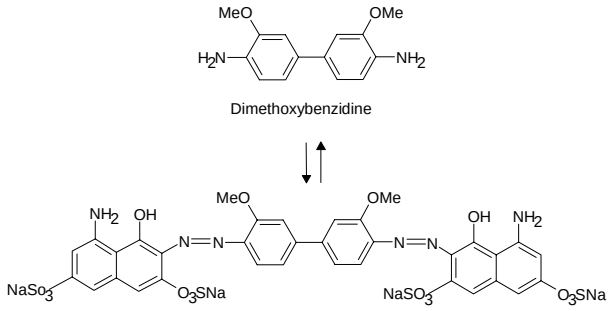


Figure 6-1. Proposed metabolic pathways of DMOB

Source: Rodgers *et al.* (1983, cited in NTP 1992)

6.2 Bacterial metabolism of DMOB-based dyes

The reductive metabolism of DMOB-based dyes (illustrated in Figure 6-2) results in the formation of DMOB. Azo reduction can result from the enzymes present in the liver or azo reductase associated with intestinal bacterial flora. Reductive cleavage of dyes metabolized to DMOB may occur, primarily, through the activity of intestinal bacteria (Martin and Kennelly 1981; Cerniglia *et al.* 1982; Brown and Dietrich 1983; Bos *et al.* 1984, 1986, all cited in NTP 1990). The free DMOB is then absorbed, resulting in systemic exposure, further metabolism (probably in the liver), and subsequent excretion.



Adapted from NTP (1990)

Figure 6-2. Formation of DMOB by reductive metabolism of C.I. Direct Blue 15

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 Blue 15, a DMOB-based dye. This study also investigated 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 (see Table 6-2) varied in the rates at which they reduced C.I. Direct Blue15.

Table 6-2. Reduction of C.I. Direct Blue 15 by various anaerobic bacteria

Organism	C.I. Direct Blue 15 reduction (nmol reduced/mg protein in 8 h)
Bacteroides thetaiotaomicron	26.5
Bifidobacterium infantis	34.8
Citrobacter sp.	110
Clostridium perfringens	315
Clostridium sp.	360
Escherichia coli	10.7
Lactobacillus acidophilus	96.1
Peptococcus anaerobius	113.6
Peptostreptococcus productus	72.7

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

The bacterial isolate from rat intestine was highly efficient in reducing C.I. Direct Blue 15 to DMOB. C.I. Direct Blue 15 (188 nmol) was added to an incubation medium containing 10^{10} bacterial cells. The mixture was assayed for DMOB and C.I. Direct Blue 15 up to 48 hours. Production of DMOB began promptly and was essentially complete (as evidenced by the absence of C.I. Direct Blue 15) within 4 hours.

6.3 Protein adduction

The covalent binding of orally administered DMOB to hemoglobin was studied in female Wistar rats. The results indicated two cleavage products, with amounts of DMOB in excess of or comparable to amounts of the monoacetyl derivative. The hemoglobin

binding index for DMOB was estimated as 2.7 (24.3 for benzidine) (Birner *et al.* 1990). This indicates a potential for binding of these residues to biological macromolecules.

6.4 Summary

The results of a number of studies of the metabolism and elimination of DMOB-based dyes provide evidence that these dyes are subject to *in vivo* metabolism giving rise to the parent amine. The metabolism of DMOB proceeds through *N*-acetylation and excretion in both urine and feces. 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. An assessment of the anaerobic metabolism of DMOB-derived dyes supports this hypothesis. Results indicate that the metabolic conversion of bisazobiphenyl dyes, based on benzidine, DMB and DMOB, to carcinogenic aromatic amines is a general phenomenon and therefore, with few exceptions, should be anticipated for each member of this class of chemicals.

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Appendix A: IARC. 1974. *Some Aromatic Amines, Hydrazine and Related Substances, N-Nitroso Compounds and Miscellaneous alkylating Agents.* 3,3′-Dimethoxybenzidine. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Lyon, France. World Health Organization. Vol. 4. A-1 – A-10.

Appendix B: IARC. 1993. *Occupational Exposures of Hairdressers and Barbers and Personal Use of Hair Colourants; Some Hair Dyes, Cosmetic Colourants, Industrial Dyestuffs and Aromatic Amines.* C.I. Direct Blue 15. IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans. Lyon, France. World Health Organization. Vol. 57. Pp. B-1 – B-14.

Appendix C: Urinary excretion of benzidine, DMB, and DMOB by dogs and rats after oral administration of dye chemicals

Table C-1. Urinary excretion of benzidine, DMB, and DMOB by dogs after oral administration of benzidine-, DMB-, and DMOB-based dyes

Benzidine based-dye	Benzidine impurity (ppm)	Dose of Benzidine as impurity (μg)	Benzidine excreted in urine during 48 hours after dosing (μg)			Percent of dose[2]
			Experiment 1	Experiment 2	Mean	
Benzidine	-	-	1161	3147	2154	0.14
Direct Black 4	3	5	320	222	271	0.08
Direct Blue 2	11	17	445	190	317	0.10
Direct Brown 2	24	36	1675	424	1049	0.24
Direct Green 1	< 5	< 8	362	455	408	0.11
Direct Orange 1	5	8	369	238	304	0.07
Direct Orange 8	34	51	545	481	513	0.11
Direct Red 28	3	5	166	316	241	0.06
DMB-based dye	**DMB impurity (ppm)**	**Dose of DMB as impurity (μg)**	**DMB excreted in urine during 48 hours after dosing (μg)**			**Percent of dose[2]**
			Experiment 1	Experiment 2	Mean	
Direct Blue 25	9	13	62	103	82	0.03
Acid Red 114	< 1	< 1.5	94	175	135	0.04
Direct Red 2	7	11	BLQ[1]	BLQ[1]	-	-
Direct Red 39	2	3	BLQ[1]	BLQ[1]	-	-
DMOB-based dye	**DMOB impurity (ppm)**	**Dose of DMOB as impurity (μg)**	**DMOB excreted in urine during 48 hours after dosing (μg)**			**Percent of dose[2]**
			Experiment 1	Experiment 2	Mean	
Direct Blue 15	46	69	61	168	114	0.03
Direct Blue 1	18	27	441	119	280	0.08

Source: Lynn *et al*. (1980).
(-) not published.
Dogs weighing 15 kg received 1.5 g of each dye (100 mg/kg). Dogs treated with benzidine received 1.5 g of the free base. Each dye was studied in 2 dogs.
[1] BLQ, below levels of quantitation but the presence of DMB was confirmed by GCMS.
[2] Percent of potential theoretical maximum produced by complete reduction of the azo bonds in the dye.

Table C-2. Urinary excretion of benzidine, DMB, and DMOB by rats after oral administration of benzidine-, DMB-, and DMOB-based dyes (100 mg/kg)

Dye	Benzidine impurity in administered dye (μg)	N-Acetylbenzidine excreted in urine during 72 hours after dosing (μg)		Percent of dose[2]
		Experiment 1	Experiment 2	
Benzidine	34,600	212 ± 34[5]		0.62 ± 0.10
Direct Black 4	< 1	22.8 ± 14.2	26.9 ± 15.3	0.26 ± 0.16
Direct Blue 2	< 1	3.1 ± 3.0	11.6 ± 4.3	0.09 ± 0.08
Direct Brown 2	< 1	18.9 ± 13.2	54.0 ± 33.1	0.34 ± 0.30
Direct Green 1	< 1	17.1 ± 13.0	13.9 ± 6.0	0.17 ± 0.11
Direct Orange 1	< 1	BLQ[1]	BLQ[1]	BLQ[1]
Direct Orange 8	< 1	17.6 ± 9.9	13.2 ± 8.5	0.13 ± 0.08
Direct Red 28	< 1	10.7 ± 7.1	12.4 ± 8.1	0.11 ± 0.07
Dye	**DMB impurity in administered dose (μg)**	**DMB excreted in urine during 72 hours after dosing (μg)[3]**		**Percent of dose[4]**
DMB	25,290	898 ± 278[6]		3.52 ± 0.99
Direct Blue 25	< 1	41 ± 3.0		0.06 ± 0.04
Acid Red 114	< 1	< 0.1		0.01
Direct Red 2	< 1	BLQ[1]		-
Direct Red 39	< 1	BLQ[1]		-
Dye	**DMOB impurity in administered dose (μg)**	**DMOB excreted in urine during 72 hours after dosing (μg)[3]**		**Percent of dose[4]**
DMOB	27250	1247 ± 145[7]		4.59 ± 0.06
Direct Blue 15	1	13.0 ± 12.9		0.17 ± 0.18
Direct Blue 1	< 1	42.5 ± 27.7		0.55 ± 0.37

Source: Lynn *et al.* (1980).
(-) not published.
Mean (± SD) daily urinary excretion of N-acetylbenzidine. Each dye was administered daily (100 mg/kg) for 10 days. Two rats were studied for each dye.
[1] BLQ, below levels of quantitation but the presence of DMB was confirmed by GCMS.
[2] Percentage of the potential theoretical maximum produced by complete reduction of the azo bonds and subsequent mono-N-acetylation.
[3] Total DMB/DMOB excreted in 72 hour following single oral dose. Mean ± SD of four animals .
[4] Percent of potential theoretical maximum produced by complete reduction of the azo bonds in the dye.
[5] Total benzidine excreted in 92 hours following single oral dose. Mean ± SD of 4 animals.
[6] Total DMB excreted in 72 hours following single oral dose. Mean ± SD of 4 animals.
[7] Total DMOB excreted in 72 hours following single oral dose. Mean ± SD of 4 animals.

Appendix D: NTP. 1990. Toxicology and Carcinogenesis Studies of DMOB Dihydrochloride in F344/N rats (Drinking Water Studies). NTP Technical Report Series TR-372. Pp. D-1 – D-68.

Appendix E: NTP. 1992. Toxicology and Carcinogenesis Studies of C.I. Direct Blue 15 in F344/N rats. NTP Technical Report Series TR-397. pp. E-1 – E-76.

Appendix F: Carcinogen Profile for DMOB and DMOB Dihydrochloride (NTP 8th Report on Carcinogens 1998)

3,3′-Dimethoxybenzidine and 3,3′-Dimethoxybenzidine Dihydrochloride CAS Nos. 119-90-4 and 20325-40-0

First Listed in the *Third Annual Report on Carcinogens*

Carcinogenicity

There is sufficient evidence for the carcinogenicity of 3,3′-dimethoxybenzidine in experimental animals (IARC V.4, 1974; IARC S.4, 1982). When administered by gavage, 3,3′-dimethoxybenzidine induced tumors at various sites including Zymbal gland tumors, intestinal carcinomas, skin carcinomas, and urinary bladder papillomas in rats of both sexes. When administered in the diet, 3,3′-dimethoxybenzidine increased the incidence of forestomach papillomas. When administered in the drinking water, 3,3′-dimethoxybenzidine dihydrochloride increased the incidence of Zymbal gland adenomas and carcinomas, liver neoplastic nodules or hepatocellular carcinomas, large intestine adenomatous polyps or adenocarcinomas, and squamous cell papillomas or carcinomas of the oral cavity in rats of both sexes; preputial gland carcinomas, basal cell adenomas and carcinomas of the skin, adenocarcinomas of the small intestine, and mesotheliomas in male rats; and clitoral gland adenomas and carcinomas, basal cell adenomas or carcinomas of the skin, mammary gland adenocarcinomas, and uterus/cervix adenomas or carcinomas in female rats (NTP 372, 1990).

An IARC Working Group reported that there is inadequate evidence for the carcinogenicity of 3,3′-dimethoxybenzidine in humans. No epidemiological data on the occurrence of cancer in workers exposed to 3,3′-dimethoxybenzidine alone appear in the literature. Most of the workers exposed to this substance were also exposed to related amines, such as benzidine, which are strongly associated with urinary bladder cancer in humans (see Benzidine, Section III.A) (IARC, V.4, 1974; IARC S.4, 1982; IARC S.7, 1987).

Properties

3,3′-Dimethoxybenzidine occurs as colorless crystals which turn violet upon standing. It is virtually insoluble in water and probably soluble in most organic solvents (e.g., ethanol, ether, acetone, benzene, and chloroform). 3,3′-Dimethoxybenzidine is available commercially as the free base (technical and 99% grades) and as its dihydrochloride salt. When heated to decomposition, 3,3′-dimethoxybenzidine emits toxic fumes of nitrogen oxides (NO_x).

Use

3,3′-Dimethoxybenzidine is used principally as a chemical intermediate for the production of azo dyes. The Society of Dyers and Colourists reported its use as an intermediate in the production of 89 dyes in 1971. Among the dyes listed were Direct Blue 218, Pigment Orange 16, Direct Blue 1, Direct Blue 15, Direct Blue 8, Direct Blue 76, and Direct Blue 98. About 30% of the 3,3′-dimethoxybenzidine consumed is used as a chemical intermediate to produce o-dianisidine diisocyanate for use in adhesive systems and as a component of polyurethane elastomers and resins. 3,3′-Dimethoxybenzidine is used as a dye for leather, paper, plastics, rubber, and textiles, and a reagent to detect metals, thiocyanates, and nitrites (IARC V.4, 1974).

Production

The Chem Sources USA directory did not identify any producers of 3,3′-dimethoxybenzidine in 1986, but listed 13 suppliers (Chem Sources, 1986). No other current production data were available. U.S. imports of 3,3′-dimethoxybenzidine were reported by the USITC to be 106,000 lb and imports of 3,3′-dimethoxybenzidine and its dihydrochloride were reported to be 655,000 lb in 1983 (USITCa, 1984). The 1979 TSCA Inventory identified two companies producing an unspecified amount of 3,3′-dimethoxybenzidine and six companies importing 55,500 lb in 1977. The CBI Aggregate was less than 1 million lb (TSCA 1979). U.S. imports of 3,3′-dimethoxybenzidine through the principal custom districts were reported to be 273,000 lb in 1971. Data on domestic production of 3,3′-dimethoxybenzidine were last reported in 1967, when five companies produced 368,000 lb; 3,3′-dimethoxybenzidine has been produced commercially for at least 50 years (IARC V.4, 1974).

Exposure

The primary routes of potential human exposure to 3,3′-dimethoxybenzidine are inhalation and dermal contact. Exposure to 3,3′-dimethoxybenzidine can occur during its use as a chemical intermediate in the production of azo dyes, o-dianisidine diisocyanate formulations, textile processing, and packaging processes. Workers potentially exposed to the chemical include dye makers and o-dianisidine diisocyanate production workers. However, present dye production processes for 3,3′-dimethoxybenzidine and its dye derivatives are generally closed systems with minimal risk to workers. The National Occupational Hazard Survey, conducted by NIOSH from 1972 to 1974, estimated that 200 workers were possibly exposed to 3,3′-dimethoxybenzidine in the workplace (NIOSH, 1976). Potential human exposure may occur as a result of the presence of trace contaminants in end products that are formulated with 3,3′-dimethoxybenzidine (e.g., azo dyes, pigments, adhesives, resins, and polyurethane elastomers). CPSC is concerned that these dyes and pigments contain residual levels or trace impurities of 3,3′-dimethoxybenzidine in the ppm range and that traces may be present in the final consumer products. Presently, no data are available on the actual quantities in the final consumer products. A dermal penetration study in rabbits indicated that a 3,3′-dimethoxybenzidine-based dye was not dermally absorbed in significant amounts.

Regulations

In late 1980, CPSC started collecting data to propose a ban on the use of 3,3′-dimethoxybenzidine-based dyes in mass-merchandised consumer dye products; however, the use of benzidine congener dyes in consumer household dyeing products and in commercial textile applications has been decreased voluntarily. Artists and crafts people have been alerted to potential hazards from inhaling powders of dyes based on 3,3′-dimethoxybenzidine. EPA regulates 3,3′-dimethoxybenzidine under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), Resource Conservation and Recovery Act (RCRA), and Superfund Amendments and Reauthorization Act (SARA). An adjustment of the statutory reportable quantity (RQ) from 1 lb to 100 lb has been established for this chemical under CERCLA. RCRA regulates 3,3′-dimethoxybenzidine as a hazardous constituent of waste. EPA has included 3,3′-dimethoxybenzidine on a list of

priority hazard substances under SARA. OSHA regulates 3,3'-dimethoxybenzidine and its dihydrochloride salt under the Hazard Communication Standard and as chemical hazards in laboratories.