ETAD

Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers

U.S. DYE MANUFACTURERS OPERATING COMMITTEE OF ETAD

January 5, 2000

Dr. C. W. Jameson
Report on Carcinogens
NIEHS
MD EC-14
79 Alexander Drive
Building 4401, Room 3127
P.O. Box 12233
Research Triangle Park, NC 27709

RE: Comments on Draft Background Documents for Nominations to the 10th Report on Carcinogens

Dear Dr. Jameson:

The United States Dye Manufacturers Operating Committee of the Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers (USOC/ETAD) submits these comments on two draft "Report on Carcinogens Background Documents" to be reviewed in conjunction with nominations to listings for the 10th *Report on Carcinogens.* The two draft Background Documents that are of interest to USOC/ETAD and are the subject of these comments are "**Dyes Metabolized to 3,3'-Dimethoxybenzidine**" and "**Dyes Metabolized to 3,3'-Dimethylbenzidine.**"

The comments contained in this letter are intended to clarify or update the information in the draft Background Documents. Specifically, USOC/ETAD seeks revision to those portions of the drafts that describe the scope of the proposed nominations and the exposure potential of the dyes at issue. As currently drafted, users of the Background Documents are likely to conclude that the proposed nominations include dyes not intended to be covered and that the extent of exposure to the dyes is far greater than is actually the case. Attached to these comments are mark-ups showing the proposed corrections to the two draft Background Documents.

Dyes in Commerce

The draft Background Document for dyes metabolized to 3,3'-dimethylbenzidine states (at page 5) that according to the Society of Dyers and Colourists "more than 95 dyes are derived from dimethylbenzidine." Presumably the source for this statement is the Colour Index, which is outdated and lists dyes that are no longer (and in some cases perhaps never were) in commerce. In fact, today there are probably less than a dozen dyes from the two classes combined in commercial use, mainly for textile applications. This is a result of the industry trend to replace many of the dimethoxybenzidine- and dimethylbenzidine-based dyes with suitable alternatives. Accordingly, the referenced

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sentence should be deleted, and in both Background Documents the extent of phase-out and substitution should be recognized.

Some of the more detailed information in the draft Background Documents is obtained from "chemical fact sheets" on DMB and DMOB posted on the web site of Spectrum Laboratories, Inc. This is not a definitive source of product use information, and the attached markups also make some changes that correct over-reliance on this source.

Furthermore, these dyes are described by NTP in its web site as"[d]yes widely used for leather, paper, plastics, rubber, and textile industries." This statement is not correct. These dyes are not widely used in any of these applications. In fact, as a result of substitution, they have become low-volume products in the aggregate, used mainly for textiles with only minor uses in other applications.

Occupational Exposure

It is correctly noted in the background documents that the primary modes of occupational exposure to dimethoxybenzidine- and dimethylbenzidine-based dyes are via the dermal or inhalational routes. However, by relying on long-outdated information, the draft Background Documents vastly exaggerate the extent of occupational exposure. The draft Background Documents report up to 60,595 workers exposed to dyes metabolized to 3,3'-dimethylbenzidine and up to 99,783 workers exposed to dyes metabolized to 3,3'-dimethoxybenzidine. These figures are derived from the 1972-1974 NIOSH National Occupational Hazard Survey and the 1981-1983 NIOSH National Occupational Exposure Survey. These long outdated surveys – concluded 25 and 16 years ago, respectively – no longer have any relevance. Since then, there has been complete change in the dye manufacturing and using industries and in the types of dyes that are marketed. In fact, our best estimates are that today there are no more than 1,000 potentially exposed workers, including production and textile mill workers, for both classes of dyes combined. The actual number is most likely even smaller. Accordingly, the outdated survey information should be deleted from the Background Documents, and the current estimate should be provided.

The draft Background Document for dimethoxybenzidine-based dyes (at page 7) states that "[c]urrent production processes using DMOB and DMOB-dyes . . . generally are closed systems that minimize worker exposure." However, no such language is included in the draft Background Document for dyes metabolized to 3,3'-dimethylbenzidine. The same production practices and exposure controls are used for both dye classes, and accordingly the same language should be added to the draft Background Document for dyes metabolized to 3,3'-dimethylbenzidine.

Nomenclature Issues

The titles and introductions to the draft Background Documents correctly state that the nominations for listings in the 10th *Report on Carcinogens* are limited to dyes metabolized to, respectively, 3,3'-dimethylbenzidine and 3,3'-dimethoxybenzidine. (In this regard, see USOC/ETAD letter, June 1, 1999, to Dr. C. W. Jameson, copy attached.) However, this distinction is lost in the rest of the draft Background

Documents, which use language such as "DMB- (or DMOB) based dyes," or dyes "derived from" the two amines. Such language is overinclusive, and therefore may be confusing to users of the documents. There are several commercially important dyes that are made from (and therefore are derived from or based on) 3,3'-dimethoxybenzidine but do not metabolize to the amine. A prime example is metal complex dyes such as C.I. Direct Blue 218, a copperized dimethoxybenzidine-based dye. This issue is very significant, because the metal complex dyes are now by far the predominant form of dyes made from these two amines.

Accordingly, in order to clarify this important issue, USOC/ETAD proposes two changes to the draft Background Documents:

(1) A clear statement at the beginning of the documents defining the terms "DMB-based dyes" and "DMOB-based dyes" to mean only dyes that are metabolized to the amine, with recognition that the metal complex dyes manufactured from 3,3'-dimethoxybenzidine are not included within the scope of the nomination or background documents.

(2) Consistent use of the terms "DMB-based dyes" and "DMOB-based dyes" throughout the documents (i.e., deletion of the terminology "derived from").

The attached markup notes the specific revisions sought by USOC/ETAD.

Background on USOC/ETAD

USOC/ETAD is the U.S. operation of the international technical organization, ETAD. ETAD is an organization of dye and organic pigment manufacturers that addresses the health, safety, and environmental issues impacting the worldwide colorants manufacturing industry. With headquarters in Basel, Switzerland, ETAD's membership presently consists of over 40 companies representing the leading colorant manufacturers in the U.S., Europe, Brazil, and Asia. The members of USOC/ETAD are BASF, Bayer, Carey Industries, Ciba, Clariant, Crompton & Knowles, DyStar, Everlight, and Fabricolor who, collectively, account for the vast majority of the domestic dye producing capacity.

USOC/ETAD member companies are committed to a rigorous product stewardship program for dyes known as DyeCare®. Through the principles of DyeCare®, USOC/ETAD members ensure that their dyes are designed, manufactured, marketed, used, recycled, and disposed of with maximum regard for health, safety, and the environment. Each company's commitment derives from leadership exerted by senior management in the company and extends to its own workers, its customers, and its suppliers.

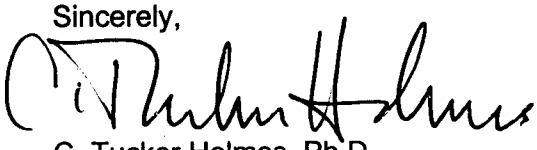
Through a written agreement with the U.S. Environmental Protection Agency, the USOC/ETAD member companies currently engaged in the production and sale of dimethoxybenzidine- and dimethylbenzidine-based dyes have committed to specific product stewardship measures, based on DyeCare®, designed to minimize exposure to these dyes. These measures include use of short drums, specific safe-handling practices, and local ventilation controls.

This practice has assured customers of a continued supply of the few remaining dimethoxybenzidine-based dyes and dimethylbenzidine-based dyes for which no suitable alternatives are available. But, at the same time, it minimizes worker exposure and ensures maximum regard for health, safety, and environmental issues.

* * * *

Thank you for this opportunity to comment on the draft background documents accompanying the nominations for listing in NTP's 10th *Report on Carcinogens*. Please direct any questions or requests for further information to me at 202-721- 4154.

Sincerely,



C. Tucker Helmes, Ph.D.
Executive Director

Attachments

File: US/INF/3

ETAD

Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers

U.S. DYE MANUFACTURERS OPERATING COMMITTEE OF ETAD

June 1, 1999

Dr. C. W. Jameson
National Toxicology Program
Report on Carcinogens
79 Alexander Drive
Building 4401, Room 3127
MD: EC-14
P. O. Box 12233
Research Triangle Park, NC 27709

RE: Comments on Proposed Listings in Tenth Report on Carcinogens

Dear Dr. Jameson:

The U. S. Dye Manufacturers Operating Committee of the Ecological and Toxicological Association of Dyes and Organic Pigment Manufacturers (USOC/ETAD) wishes to submit these comments on the substances to be reviewed in 1999 for listing in the tenth edition of the Report on Carcinogens (FR 64, 15983, April 2, 1999; FR 64, 19188, April 19, 1999). The substances nominated by NTP to be reviewed for listing in the tenth report that are of particular concern to USOC/ETAD, as shown in the April 2, 1999 Federal Register notice, are "Dyes Metabolized to Dimethoxybenzidine (Dimethoxybenzidine Dyes as a Class)" and "Dyes Metabolized to Dimethoxybenzidine (Dimethoxybenzidine Dyes as a Class)."

First, USOC/ETAD assumes that it is not the intention of the NTP to review Dimethoxybenzidine dyes twice, but rather to review Dimethoxybenzidine dyes as a class and Dimethylbenzidine dyes as a class. Furthermore, to be precise, the positions of the methoxy and methyl substituents on the benzidine ring system should be specified. For the Dimethoxybenzidine dyes, the positions on the ring should be specified as 3,3'-Dimethoxybenzidine (i.e., *o*-dianisidine) and for the Dimethylbenzidine dyes, the positions should be specified as 3,3'-Dimethylbenzidine (i.e., *o*-tolidine).

It is our understanding from discussions with NTP program staff that the NTP proposes to limit the review of these two classes of dyes to only dyes that are defined as dyes that could be metabolized to either 3,3'-Dimethoxybenzidine (CAS # 119-90-4) or 3,3'-Dimethylbenzidine (CAS # 119-93-7). USOC/ETAD concurs with this definition and agrees with NTP that copperized Dimethoxybenzidine dyes, such as C.I. Direct Blue 218 (CAS # 28407-37-6), should be excluded from the scope of review because they can not be metabolized to the free amine.

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In conclusion, USOC/ETAD asks that NTP correct its list of substances nominated to be reviewed for listing in the tenth edition of the Report on Carcinogens to read as follows:

"Dyes Metabolized to 3,3'-Dimethoxybenzidine (CAS # 119-90-4) (3,3'-Dimethoxybenzidine Dyes as a Class)" and

"Dyes Metabolized to 3,3'-Dimethylbenzidine (CAS # 119-93-7) (3,3'-Dimethylbenzidine Dyes as a Class)."

USOC/ETAD is the U.S. operation of the international technical organization, ETAD. ETAD is an organization of dye and organic pigment manufacturers that addresses the health, safety, and environmental issues impacting the worldwide colorants manufacturing industry. With headquarters in Basel, Switzerland, ETAD's membership presently consists of 41 companies representing the leading colorant manufacturers in the U.S., Europe, Brazil, and Asia. Members of USOC/ETAD are BASF, Bayer, Carey Industries, Ciba, Clariant, Crompton & Knowles, DyStar, Everlight, Fabricolor, and Sunbelt who, collectively, account for the vast majority of the domestic dye producing capacity.

Thank you for this opportunity to comment on the substances being considered for listing in NTP's tenth edition of the Report on Carcinogens. Please direct any questions or requests for further information to the undersigned at 202-721-4154.

Sincerely,

C. Tucker Helmes, Ph.D.
Executive Director

File: US/INF/3

Draft

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:
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
Contract Number NOI-ES-85421

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

with the exception of metal complex dyes, which are excluded from this classification,

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).

(excluding metal complex dyes)

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.

("DMOB-based dyes"). Metal complex dyes, however, do not metabolize to the amine and are excluded from this classification.

2 Human Exposure

2.1 Use

The major use of DMOB is as an intermediate in the production of DMOB-based dyes, which are mainly 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). There has been widespread substitution of DMOB-based dyes by other products, and only a few remain in commercial use.

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

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-based 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 only national estimates of exposure to DMOB and DMOB-based dyes are the outdated

~~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, and 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

when more DMOB-based dyes were in commerce. Current industry estimates are that there are fewer than 1,000 potentially-exposed workers to DMOB-based dyes and DMB-based dyes combined.

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.

DMOB-based

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,

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~~ based 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.

Draft

Report on Carcinogens Background Document for

Dyes Metabolized to 3,3′-Dimethylbenzidine

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

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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

several

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

Table 2-4. OSHA Regulations 9
Table 3-1. Cohort studies of workers exposed to DMB 13
Table 4-1. C.I. acid red 114 consumption by male and female F-344/N rats 15
Table 4-2. Tumor incidences in F344/N rats administered C.I. Acid Red 114 in drinking water for up to 104 weeks 16
Table 4-3. Average weekly doses of DMB dihydrochloride administered to BALB/c mice for up to 104 weeks 18
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 19
Table 4-5. Incidence of treatment-related tumors in F344/N rats dosed with 0 or 150 ppm of DMB for 9 months 20
Table 4-6. Tumor incidences in F344/N rats administered DMB hydrochloride in drinking water for 14 months 21
Table 4-7. Detection of activated oncogenes in spontaneous tumors and tumors induced by DMB or a DMB-~~derived~~ based dye 22
Table 4-8. Identity and frequency of activated *ras* genes within specific types of induced and spontaneous tumors 23
Table 4-9. Qualitative tumor responses to DMB, DMB-based dye, DMOB, and DMOB-based dye administered in the drinking water to rats 24
Table 6-1. Urinary excretion of DMB by dogs after oral administration of DMB-based dyes (100 mg/kg) 31
Table 6-2. Urinary excretion of DMB by rats after oral administration of DMB or DMB-based dyes (100 mg/kg) 32
Table 6-3. Reduction of C.I. Direct Red 2 by various anaerobic bacteria 34

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.

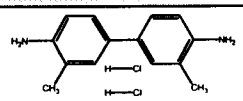
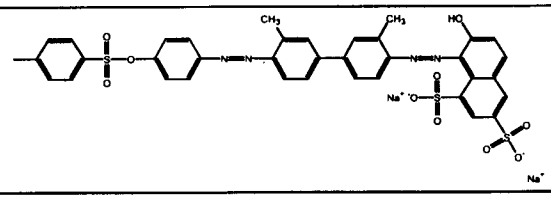
DMB ($C_{14}H_{16}N_2$, mol wt 212.29, CASRN 119-93-7) is a methylated congener of benzidine and also is known by the following names (Chemfinder 1999):

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

("DMB-based dyes")

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	

2 Human Exposure

2.1 Use

The major 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 also biological stains, and in color photography. ~~Approximately 20% of~~ DMB is also used in the production of polyurethane-based high-strength elastomers, coatings, and rigid plastics, ~~DMB~~ and ~~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). There has been widespread substitution of DMB-based dyes by other products, and only a few remain in commercial use.

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).

Current production practices using DMB and DMB-based dyes, however, generally are closed systems that minimize worker exposure.

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).

Lately national estimates of exposure to DMB and DMB-based dyes are the outdated,

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).

and

from

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).

when more DMB-based dyes were in commerce. Current industry estimates are that there are fewer than 1,000 potentially-exposed workers to DMB-based dyes and DMOB-based dyes combined.

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~~ based 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~~ based dye

Treatment/Tumor type	Frequency (Positive/tested)	Transformation efficiency, focl 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.

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~~based 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