3,3'-Dichlorobenzidine-based Compounds Metabolized to 3,3'-Dichlorobenzidine

3,3'-Dichlorobenzidine-based compounds metabolized to 3,3'-dichlorobenzidine are primarily colorants used in printing inks, paints, textiles, paper, plastic and rubber. These colorants are primarily yellow, orange, and red hues used in dyes (which are soluble in water or organic solvents) and pigments (which are not soluble). They include 18 pigments (*e.g.*, Pigment Yellow 12, Pigment Yellow 13, Pigment Yellow 14, Pigment Yellow 17, Pigment Yellow 83, Pigment Orange 13, Pigment Orange 34, and Pigment Red 38) and four dyes (*e.g.*, Direct Red 46).

Exposure to 3,3'-dichlorobenzidine-based compounds metabolized to 3,3'-dichlorobenzidine may occur to workers who manufacture or use these compounds. The extent to which the general public may be exposed as a result of consumer product use or environmental contamination is unclear.

3,3'-Dichlorobenzidine is a Proposition 65 carcinogen listed in 1987, and has been identified by IARC as a Group 2B carcinogen (IARC, 1987), and by NTP in 1981 as "reasonably anticipated to be a human carcinogen" (NTP, 2011). Given this, 3,3'-dichlorobenzidine-based compounds metabolized to 3,3'-dichlorobenzidine passed the animal data screen, underwent a preliminary toxicological evaluation, and are being brought to the Carcinogen Identification Committee for consultation. This is a compilation of the relevant studies identified during the preliminary toxicological evaluation.

Epidemiological data

One cohort study of male U.S. manufacturing workers exposed to mixtures of benzidine and 3,3'-dichlorobenzidine was identified (Rosenman and Reilly, 2004). An increased risk of leukemia and bladder cancer was observed in exposed workers.

No cancer epidemiology studies on 3,3'-dichlorobenzidine-based compounds metabolized to 3,3'-dichlorobenzidine were identified.

Animal carcinogenicity data

3,3'-Dichlorobenzidine induced tumors in animal carcinogenicity studies in multiple species at multiple tumor sites, as reviewed by IARC (2010, pp. 242, 209-213)

- Administration in the diet in male mice (hepatoma)
- Administration in the diet in male rats (granulocytic leukemia, Zymbal gland carcinoma)

- Administration in the diet in male and female rats (mammary gland adenocarcinoma)
- Administration in the diet in female dogs (transitional cell carcinoma of the urinary bladder and hepatocellular carcinoma)
- Transplacental exposure in mice (lymphoid leukemia)

Pigment Yellow 12 (Diarylanilide Yellow)

- 78-week diet studies (and observed for additional 28 weeks) in male and female F344 rats: NCI (1978)
    - *No treatment-related tumor findings*

- 78-week diet studies (and observed for additional 47 weeks) in male and female B6C3F_1 mice: NCI (1978)
    - *No treatment-related tumor findings*

Other relevant data

- Structure activity considerations (Tables 1 and 2)
    - Benzidine and the benzidine congeners 3,3'-dichlorobenzidine, 3,3'-dimethoxybenzidine, and 3,3'-dimethylbenzidine share close structural similarities.
    - Benzidine and the benzidine congeners 3,3'-dichlorobenzidine, 3,3'-dimethoxybenzidine, and 3,3'-dimethylbenzidine are carcinogens identified by Proposition 65, IARC, and NTP.
    - Benzidine and the benzidine congeners 3,3'-dichlorobenzidine, 3,3'-dimethoxybenzidine, and 3,3'-dimethylbenzidine have each been used in the production of azo colorants. [R-N=N-R' is the chemical formula for a compound containing one azo bond.] The azo bond can be cleaved reductively by intestinal bacteria or azo reductases in the liver or other tissues to release the carcinogenic aromatic amine.
    - Compounds metabolized to benzidine (*e.g.*, benzidine-based dyes) are carcinogens identified by Proposition 65, IARC, and NTP.
    - Compounds metabolized to 3,3'-dimethoxybenzidine (*e.g.*, 3,3'-dimethoxybenzidine-based dyes metabolized to 3,3'-dimethoxybenzidine) are carcinogens identified by Proposition 65 and NTP.
    - Compounds metabolized to 3,3'-dimethylbenzidine (*e.g.*, 3,3'-dimethylbenzidine-based dyes metabolized to 3,3'-dimethylbenzidine) are carcinogens identified by Proposition 65 and NTP.

Table 1. Chemical structures of benzidine and benzidine congeners

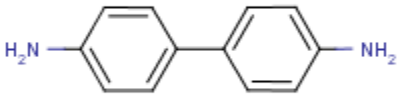
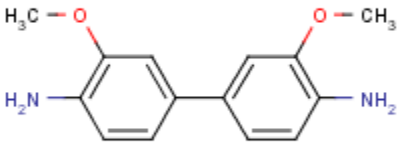
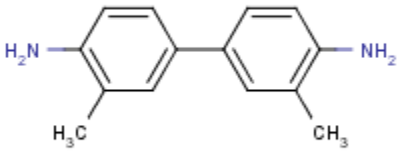
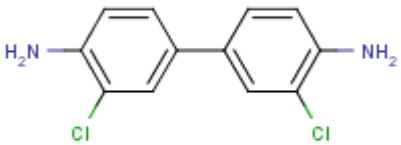
	Benzidine
	3,3'-Dimethoxybenzidine
	3,3'-Dimethylbenzidine
	3,3'-Dichlorobenzidine

Table 2. Benzidine- and benzidine congener-based compounds identified as carcinogens

Chemical	Proposition 65	IARC[1]	NTP[2]
3,3'-Dichlorobenzidine	Listed (1987)	Group 2B (1987)	Reasonably anticipated (1981)
3,3'-Dichlorobenzidine dihydrochloride	Listed (1998)	--	Reasonably anticipated (1981)
3,3'-Dichlorobenzidine-based compounds metabolized to 3,3'-dichlorobenzidine	*Subject of current review*	--	--
Benzidine [and its salts]	Listed (1987)	Group 1 (1982)	Known (1980)
Benzidine-based dyes / Dyes metabolized to benzidine	Listed (1992)	Group 2A (1987) Group 1 (2010)	Known (2000)
3,3'-Dimethoxybenzidine (*ortho*-Dianisidine)	Listed (1988)	Group 2B (1987)	Reasonably anticipated (1983)
3,3'-Dimethoxybenzidine-based dyes metabolized to 3,3'-dimethoxybenzidine	Listed (2004)	---	Reasonably anticipated (2002)
3,3'-Dimethylbenzidine (*ortho*-Tolidine)	Listed (1988)	Group 2B (1987)	Reasonably anticipated (1983)
3,3'-Dimethylbenzidine-based dyes metabolized to 3,3'-dimethylbenzidine	Listed (2004)	--	Reasonably anticipated (2002)

[1] IARC (2011)
[2] NTP (2011)

- Metabolism of 3,3'-dichlorobenzidine-based compounds to 3,3'-dichlorobenzidine *in vivo*
    - 3,3'-Dichlorobenzidine detected in the urine of rabbits administered a single gavage dose of Benzidine Yellow (Pigment Yellow 13): Akiyama (1970)
    - 3,3'-Dichlorobenzidine detected in the urine of male Fischer 344 rats administered a single gavage dose of Direct Red 46: Bowman *et al.* (1983)

- Hemoglobin adducts derived from 3,3'-dichlorobenzidine measured *in vivo* in female Wistar rats after 4-week oral administration of
    - Direct Red 46
        - *positive*: Zwirner-Baier and Neumann (1994)
        - *positive*: Sagelsdorff *et al.* (1996)
    - Pigment Yellow 17
        - *positive*: Zwirner-Baier and Neumann (1994)
        - *negative*: Sagelsdorff *et al.* (1996)
    - Pigment Yellow 13
        - *inconclusive*: Sagelsdorff *et al.* (1996)

- Genotoxicity, as reviewed in ATSDR (2001, pp. 42-50; 1998, pp. 52-56), CCRIS (2008a, 2008b) and GENE-TOX (1995, 1998a, 1998b). See Table 3.

Table 3. Genotoxicity of benzidine and benzidine congeners

Chemical	**Benzidine (BZ)** *(ATSDR, 2001, CCRIS 2010)*	**3,3'-Dimethoxy-BZ** *(CCRIS, 2008a; GENETOX, 1998b)*	**3,3'-Dimethyl-BZ** *(CCRIS, 2008b; GENETOX, 1998a)*	**3,3'-Dichloro-BZ** *(ATSDR, 1998)*
Mutations in *Salmonella*	+	+	+	+
Mutations in mammalian cells	+	NT	+	NT
CA *in vitro*	+	+	+	NT
SCE *in vitro*	+	+	+	NT
UDS *in vitro*	+	+	+/-	+
MN *in vivo*	+	NT	NT	+
SCE *in vivo*	NT	NT	NT	+
UDS *in vivo*	+	NT	NT	+
DNA binding *in vivo*	+	NT	NT	+
Cell transformation	+	NT	+	+

CA: chromosome aberration; SCE: sister chromatid exchange; UDS: unscheduled DNA synthesis; MN: micronucleus; NT: not tested

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[1] Excerpts or the complete publication have been provided to members of the Carcinogen Identification Committee, in the order in which they are discussed in this document.

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