

# BENZIDINE

Benzidine was considered by previous IARC Working Groups in 1987 and 2008 ([IARC, 1987, 2010](#)). Since that time new data have become available, which have been incorporated in this *Monograph*, and taken into consideration in the present evaluation.

## 1. Exposure Data

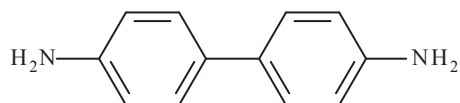
### 1.1 Identification of the agents

#### 1.1.1 Benzidine

*Chem. Abstr. Serv. Reg. No.:* 92-87-5

*Chem. Abstr. Serv. Name:*

[1,1'-Biphenyl]-4,4'-diamine



$C_{12}H_{12}N_2$

Relative molecular mass: 184.24

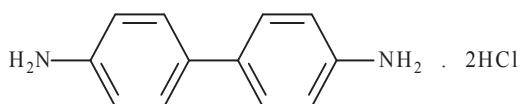
*Description:* White to slightly reddish, crystalline powder; darkens on exposure to air and light ([O'Neil, 2006](#)).

*Solubility:* Slightly soluble in water, diethyl ether, and dimethyl sulfoxide (DMSO); soluble in ethanol ([Lide, 2008](#))

#### 1.1.2 Benzidine dihydrochloride

*Chem. Abstr. Serv. Reg. No.:* 531-85-1

*Chem. Abstr. Serv. Name:* [1,1'-Biphenyl]-4,4'-diamine, dihydrochloride



$C_{12}H_{10}N_2 \cdot 2HCl$

Relative molecular mass: 257.18

*Description:* Crystalline solid ([O'Neil, 2006](#))

*Solubility:* Soluble in water and alcohol ([O'Neil, 2006](#))

### 1.2 Uses

Benzidine has been used since the 1850s as the reagent base for the production of a large number of dyes, particularly azo dyes for wool, cotton, and leather ([IARC, 2010](#)). In the past, benzidine also has been used in clinical laboratories for detection of blood, as a rubber compounding agent, in the manufacture of plastic films, for detection of hydrogen peroxide in milk, and for quantitative determination of nicotine. Most of these uses have been discontinued because of toxicological concerns. Some dyes used as stains for microscopy and similar laboratory applications may contain benzidine as an impurity ([ATSDR, 2001; NTP, 2005](#)).

Manufacture of benzidine is prohibited in several individual countries (e.g. Japan, Republic of Korea, Canada, and Switzerland) and in Europe through European Union (EU) legislation. It has not been manufactured on a large scale for commercial purposes in the United

**Table 1.1 Estimated numbers of workers exposed to benzidine in the European Union**

Industry, occupational activity	
Manufacture of textiles	160
Education services	3700
Research and scientific institutes	1900
Medical, dental, other health & veterinary services	1300
TOTAL	6900

From: [CAREX \(1999\)](#)

States of America (USA) since 1976, although small quantities remain available for diagnostic testing. It is reportedly produced and/or supplied in research quantities in Germany, Hong Kong Special Administrative Region, India, the People's Republic of China (China), Switzerland, and the USA ([IARC, 2010](#)). Production and use of benzidine in dye production has been reported in some developing countries, as has the transfer of benzidine production from other European countries to the former Serbia and Montenegro and to the Republic of Korea ([Carreón et al., 2006a](#)).

In 1994, the German Government prohibited the use of certain azo dyes in consumer goods that come in direct, prolonged contact with human skin (e.g. clothing, bedding, footwear, gloves, etc.). The dyes affected are those that, after reduction of one or more azo groups, may release one or more of 20 specific aromatic amines (including benzidine) in detectable concentrations (i.e. > 30 parts per million (ppm)). In 2002, a EU Directive (76/769/EEC) expanded coverage to articles that come in contact with the oral cavity and added two amines to the list ([Ahlström et al., 2005](#); [ETAD, 2008](#)). The US Food and Drug Administration limits benzidine content in food colourants to 1 part per billion (ppb). While exposure via ingestion is considered highly unlikely, other impurities in synthetic colouring agents may be metabolized to benzidine after ingestion ([ATSDR, 2001](#)).

## 1.3 Human exposure

### 1.3.1 Occupational exposure

Estimates of numbers of workers potentially exposed to benzidine have been published by CAREX (CARcinogen EXposure) in Europe. CAREX is an international information system that provides selected exposure data and documented estimates of the number of exposed workers by country, carcinogen, and industry ([Kauppinen et al., 2000](#)). Based on data on occupational exposure to known and suspected carcinogens collected from 1990 to 1993, it is estimated from the CAREX database that 6900 workers were exposed to benzidine in the European Union. [Table 1.1](#) presents the results for benzidine by industry in the EU ([CAREX, 1999](#)).

[NIOSH \(1984\)](#) estimated from the US National Occupational Exposure Survey (1981–83) that 1554 workers (including 426 women) were potentially exposed to benzidine and 2987 workers (2464 women) to benzidine dihydrochloride.

Studies that reported airborne or urinary concentrations and results of dermal wipes of benzidine or benzidine derivatives in the benzidine-based dye industry have been reviewed ([IARC, 2010](#)).

### 1.3.2 Non-occupational exposure

Because benzidine may be produced only for captive consumption (i.e. in-house by the producer only, in closed systems with stringent controls), its direct release into the environment is expected to be low.

Benzidine-based dyes can contain various amounts of benzidine as a contaminant. The general population can be exposed to benzidine when in contact with consumer goods containing benzidine or benzidine based-dyes such as leather products ([Ahlström et al., 2005](#)), clothes and toys ([Garrigós et al., 2002](#)). Some food colourants such as tartrazine and sunset yellow FCF have been

reported to contain trace amounts of benzidine (< 5 to 270 ng/g) ([Lancaster & Lawrence, 1999](#)).

## 2. Cancer in Humans

In a previous *IARC Monograph* ([IARC, 2010](#)) it was concluded that there is *sufficient evidence* in humans for the carcinogenicity of benzidine in the human bladder. Numerous case reports from different countries have been published ([IARC, 1972, 1982, 1987, 2010](#)). In one extreme instance, all five of a group of workers permanently employed in the manufacture of benzidine for 15 years or more developed bladder cancer ([IARC, 1982](#)). [Vigliani & Barsotti \(1962\)](#) reported on 20 workers with tumours of the urinary bladder between 1931 and 1948 among 83 Italian dyestuff workers involved in benzidine production and use. [Case et al. \(1954\)](#) found 10 bladder-cancer deaths among dyestuff workers exposed only to benzidine (standardized mortality ratio (SMR) 13.9 [95%CI: 6.7–25.5]). In benzidine-exposed workers in the chemical dye industry in China, the morbidity from bladder cancer increased with increasing duration of exposure ( $p$  for trend < 0.01) ([Sun & Deng, 1980](#)), while in a cohort of benzidine manufacturers in the USA, risks were significantly elevated for those with  $\geq 2$  years exposure to benzidine (SMR 13.0 [95%CI: 4.8–28.4]) ([Meigs et al., 1986](#)). In a cohort of dyestuff workers in Torino, Italy, the SMR was 100.8 [95%CI: 60.8–167.2] during exposure and 14.8 [95%CI: 71–31.0] at 20 or more years after exposure ceased ([Piolatto et al., 1991](#)). In a study of workers from a chemical manufacturing plant in Shanghai, China, an interaction was found between benzidine exposure and cigarette smoking in the development of bladder cancer ([Wu, 1988](#)). Relative to those who did not smoke and had no exposure to benzidine, the risks (RR) for bladder cancer were 6.2 ( $P = 0.05$ ) for smokers who were not exposed to benzidine;

63.4 ( $P < 0.05$ ) for non-smokers exposed to benzidine; and 152.3 ( $P < 0.01$ ) for smokers exposed to benzidine. In another study of Chinese workers in benzidine production and use facilities, the odds ratios (OR) for bladder cancer were 1.0, 2.7 (1.1–6.3) and 4.4 (1.8–10.8) for low, medium and high cumulative exposure to benzidine, respectively, after adjustment for life-time cigarette smoking ([Carreón et al., 2006b](#)). In a case-control study in Canada, excesses of renal cell cancer in relation to duration of exposure to benzidine ( $P < 0.004$ ) were noted, but other consistently supporting data were not found ([Hu et al., 2002](#)).

Overall, case reports and epidemiological investigations from several countries show strong and consistent associations between benzidine exposure and risk for bladder cancer.

## 3. Cancer in Experimental Animals

Studies on the carcinogenicity of benzidine in the mouse, rat, hamster, rabbit, dog, and frog by oral, subcutaneous injection, intraperitoneal injection, or inhalation routes of exposure have been reviewed by previous IARC Working Groups ([IARC, 1972, 1982, 1987, 2010](#)). There have been no additional carcinogenicity studies in animals reported since the most recent evaluation ([IARC, 2010](#)). Results of adequately conducted carcinogenicity studies are summarized in [Table 3.1](#).

Benzidine and its dihydrochloride were adequately tested for carcinogenicity by oral administration (feed, drinking-water or gavage) in eight experiments in mice and one experiment in rats; by subcutaneous injection in one experiment in rats and one experiment in frogs; and in rats in one experiment by intraperitoneal injection.

Following oral administration to male and female mice, newborn and adult, of different strains, benzidine significantly increased the

**Table 3.1 Carcinogenicity studies of benzidine in experimental animals**

Species, strain (sex)	Route	Incidence of tumours	Significance	Comments
<b>Duration</b> <b>Reference</b>	<b>Dosing regimen,</b> <b>Animals/group at start</b>			
Mouse, B6C3F <sub>1</sub> (M) 90 wk <a href="#">Vesselinovitch et al. (1975)</a>	Feed Four groups of 50 M mice were fed 150 ppm benzidine as the dihydrochloride (certified ACS grade) in the diet for 45 wk. Groups of 50 mice were sacrificed at 45, 60, 75, and 90 wk of age. A group of 100 male mice that were sacrificed at 90 wk of age served as untreated controls	Hepatocellular carcinomas: 1/98, 2/50, 5/50 <sup>s</sup> , 14/50 <sup>s</sup> , 24/50 <sup>s</sup> Hepatocellular adenomas: 1/98, 6/50 <sup>s</sup> , 15/50 <sup>s</sup> , 17/50 <sup>s</sup> , 11/50 <sup>s</sup>	*[P < 0.0001], §[P < 0.05]	
Mouse, B6C3F <sub>1</sub> (M) 90 wk <a href="#">Vesselinovitch et al. (1975)</a>	Feed Three groups of 50 M mice were fed 150 ppm benzidine as the dihydrochloride salt (certified ACS grade) in the diet for 39, 54, or 84 wk. All animals were sacrificed at 90 wk of age. A group of 100 M mice that were sacrificed at 90 wk of age served as untreated controls.	Hepatocellular tumours [benign and malignant]: 1/98, 35/50 <sup>s</sup> , 25/50 <sup>s</sup> , 44/50 <sup>s</sup>	*[P < 0.0001]	
Mouse, B6C3F <sub>1</sub> (M, F) 90 wk <a href="#">Vesselinovitch et al. (1979)</a>	Feed Groups of 43–65 M and F offspring mice were fed and/or exposed to a diet containing 150 ppm benzidine as the dihydrochloride (1) through fed mothers from the 12th d of gestation (prenatal) to delivery; (2) through fed mothers with litters from delivery to weaning; (3) to offspring from weaning to 90 wk of age; (4) during the pre-natal and pre-weaning period; or (5) prenatally, during pre-weaning and in adulthood. Groups 98 M and 100 F mice served as untreated controls.	Hepatocellular tumours [benign and malignant]: Group 1–17/55 <sup>s</sup> (M), 2/62 (F) Group 2–62/65 <sup>s</sup> , 2/43 Group 3–25/44 <sup>s</sup> , 48/50 <sup>s</sup> Group 4–49/49 <sup>s</sup> , 12/48 <sup>s</sup> Group 5–50/50 <sup>s</sup> , 47/50 <sup>s</sup> Controls–1/98, 0/100	*[P < 0.0001]	Age NR Purity NR
Mouse, B6C3F <sub>1</sub> (M, F) 90 wk <a href="#">Vesselinovitch (1983)</a>	Feed Groups of pregnant F or weanling M + F B6C3F <sub>1</sub> mice were fed diets containing 150 ppm of benzidine as the dihydrochloride: group 1–prenatal exposure (12th d of gestation to delivery) group 2–pre-weaning exposure (delivery to weaning) group 3–from weaning to 90 wk Groups of 98 M + 96 F mice were sacrificed at 90 wk and served as controls.	Hepatocellular adenomas and carcinomas: Group 1: 8/36 <sup>s</sup> (M), 2/56 (F) Group 2: 35/52 <sup>s</sup> , 9/43 <sup>s</sup> Group 3: 22/26 <sup>s</sup> , 16/25 <sup>s</sup> Controls: 1/98, 0/96 Hepatocellular carcinomas: Group 1: 3/36 <sup>s</sup> (M), 1/56 (F) Group 2: 26/52 <sup>s</sup> , 5/43 <sup>s</sup> Group 3: 17/26 <sup>s</sup> , 16/25 <sup>s</sup> Controls: 0/98, 0/96	*[P < 0.05], §[P < 0.0001]	Liver was the only tissue examined. Purity NR

Table 3.1 (continued)

Species, strain (sex)	Route	Incidence of tumours	Significance	Comments
Mouse, F <sub>1</sub> (C57BL/6Jf C3Hf/Nctr females × BALB/cStCrLfc3Hf/Nctr males) and mono-hybrid (F <sub>1</sub> females and F <sub>1</sub> males) up to 80 wk <a href="#">Nelson et al. (1982)</a>	Drinking-water Groups of M + F mice (F <sub>1</sub> ) and MC mice were given drinking-water containing 0, 30, 60, 120, 200, or 400 ppm of benzidine as the dihydrochloride. Groups of mice were sacrificed after 40, 60, or 80 wk of treatment.	Hepatocellular adenomas and carcinomas: F <sub>1</sub> M, 40 wk-0/49, 0/98, 0/72, 0/51, 3/50, 1/28 F <sub>1</sub> F, 40 wk-0/48, 2/98, 1/72, 0/49, 5/50, 13/29 F <sub>1</sub> M, 60 wk-1/48, 0/73, 4/49, 9/48, 9/47, 12/23 F <sub>1</sub> F, 60 wk-1/48, 3/74, 4/52, 24/58, 54/61, 41/41 F <sub>1</sub> M, 80 wk-0/46, 5/44, 6/47, 13/45, 8/21, 16/20 F <sub>1</sub> F, 80 wk-0/47, 9/43, 23/43, 34/37, 9/9, 0/1 MC M, 40 wk-0/50, 1/101, 0/71, 1/48, 0/52, 1/27 MC F, 40 wk-0/48, 0/97, 0/72, 3/51, 6/50, 10/26 MC M, 60 wk-0/48, 3/69, 3/46, 8/50, 8/43, 7/26 MC F, 60 wk-1/48, 7/72, 12/54, 26/56, 47/60, 33/38 MC M, 80 wk-2/45, 2/41, 7/43, 14/44, 7/19, 11/17 MC F, 80 wk-0/48, 12/43, 20/42, 31/32, 7/8, 5/6	No statistics provided [significant for many groups]	Age NR Purity NR Initial number of animals unclear
Mouse, F <sub>1</sub> (C57BL/6Jf C3Hf/Nctr females × BALB/cStCrLfc3Hf/Nctr males) and mono-hybrid (F <sub>1</sub> females and F <sub>1</sub> males) 33 mo (Lifetime) <a href="#">Littlefield et al. (1983, 1984)</a>	Drinking-water Groups of 72-120 M + F mice (F <sub>1</sub> ) and MC mice were given drinking-water containing 0, 30, 40, 60, 80, 120, or 160 ppm (M) and 0, 20, 30, 40, 60, 80, or 120 ppm (F) of benzidine as the dihydrochloride.	Hepatocellular carcinomas: F <sub>1</sub> M: 14/125, 24/119, 30/96, 23/71, 35/71, 51/71, 49/71 F <sub>1</sub> F: 3/124, 51/120, 52/95, 45/72, 55/71, 60/69, 64/72 MC M: 17/123, 20/118, 20/95, 23/72, 24/71, 37/71, 32/71 MC F: 10/125, 54/119, 43/95, 31/71, 37/72, 51/69, 56/72 The authors also noted that a dose effect was observed for Harderian gland adenomas in F <sub>1</sub> and MC males (P = 0.02) and females (P = 0.02) and for angiomas of the uterus in F <sub>1</sub> and MC females (P = 0.07).	Authors indicate that for all four strain/sex combinations, there was a significant dose-related trend for fatal liver tumours, incidental liver tumours, and the pooled estimate using Peto's test, however details on statistics were not provided.	Purity NR
Mouse, B6C3F <sub>1</sub> (M, F) 90 wk <a href="#">Vesselinovitch et al. (1975)</a>	Gavage Groups of 75 M and 75 F mice were given 0.5 or 1.0 mg benzidine as the dihydrochloride salt twice weekly. All animals were sacrificed at 90 wk of age. Groups of 100 M and 100 F mice served as untreated controls, killed at 90 wk.	Hepatocellular tumours [benign and malignant]: M-1/98, 3/75, 12/75* F-0/100, 4/75*, 17/75*	No statistics provided * [P < 0.0005], * [P < 0.05]	Purity NR Vehicle NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, ICR (F) 63 wk <a href="#">Miyakawa &amp; Yoshida (1980)</a>	Feed Groups of F mice were implanted with a 45-mg glass bead in the urinary bladder and divided into three groups: 30 were fed basal diet; 60 were fed diet containing 0.2% benzidine; and 60 were fed diet containing a mixture of 0.2% benzidine and 2% DL-tryptophan. Treated groups received diets starting at six wk of age for 20 wk and were then fed the control diet for 40–43 wk.	Hepatomas [adenomas]: 0/30, 34/41*, 24/51 No bladder tumours were found in any of the animals.	* $P < 0.01$ , compared with control and tryptophan treated groups	Purity NR
Mouse, Strain NR (M, F) Lifetime <a href="#">Bonser et al. (1956)</a>	Subcutaneous Injection Groups of 30 M and 30 F mice received 0.1 ml of a freshly made 3% solution of benzidine (purity NR) in arachis oil by subcutaneous injection, twice/wk for 50 wk, and were then observed for life. Groups of 30 M and 30 F mice served as vehicle controls.	Hepatomas: M–0/30, 4/30; F–0/30, 3/30	No statistics provided	Age NR
Rat, Sprague-Dawley (F) 9 mo <a href="#">Griswold et al. (1968)</a>	Gavage Four groups of 10–20 F rats were given benzidine at doses of 12, 25, 35 or 50 mg/rat in sesame oil by stomach tube, daily for 30 d, and sacrificed after a 9-mo observation period. A group of 140 F rats served as vehicle controls.	Mammary carcinomas: 3/132, 5/10*, 7/19*, -, 4/5*	No statistics provided * [ $P < 0.0001$ ]	Small number of animals per group, high mortality in top 2 dose groups. Purity NR
Rat, Sherman (M, F) Lifetime <a href="#">Spitz et al. (1950)</a>	Subcutaneous injection Groups of 45 to 155 M and F rats were injected with 15 mg/rat of “technical” benzidine, “pure” benzidine, or benzidine sulfate, dissolved in 1 mL of olive oil once/wk throughout life. A group of 50 rats served as vehicle controls.	Hepatomas: Technical–8/78 (M), 0/155 (F); Pure–5/45 (M), 1/107 (F); Sulfate–not tested (M), 1/153 (F) External auditory canal carcinomas (M+F): Technical –54/233*; Pure –32/152*; Sulfate –16/153* None of the above tumours were reported in the 50 control animals.	No statistics provided * [significant]	Survival at 300 d was 56% for controls and 15% to 3% for treated rats. Poor survival due to heat (no air conditioning in animal rooms). Sex of control animals NR

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
Rat, CD (F) 46 wk <a href="#">Morton et al. (1981)</a>	Intraperitoneal injection Three groups of 30 F rats were given injections of 0, 10, or 30 µmol/kg bw benzidine in trioctanoin suspensions, twice/wk for 4 wk.	Mammary gland tumours (all): 3/30, 7/30, 12/29* Mammary gland adenocarcinomas: 1/30, 2/30, 7/29** Zymbal gland tumours (benign and malignant): 1/30, 1/30, 7/29** No tumours of the liver were found.	* $P < 0.01$ ** $P < 0.05$	Purity NR
Frog, <i>Rana temporaria</i> 38 wk <a href="#">Khudoley (1977)</a>	Subcutaneous injection A group of 37 frogs (1–1.5 yr old) received a subcutaneous injection, once/wk, of 0.2–0.5 mL of a 0.5% solution of benzidine in mineral oil, for up to 38 wk (total dose, 45–114 mg/animal). A group of 67 frogs given subcutaneous injections of 0.2–0.5 mL mineral oil once weekly for 42 wk served as vehicle controls.	Liver tumours: 0/67, 3/14* Tumours of the haematopoietic system: 0/67, 4/14*	* $[P < 0.01]$	Fourteen animals in the treated group were still alive when the first tumour appeared at 16 wk. The high mortality was the result of exceeding the maximum tolerated dose. Sex NR. Purity NR. Histopathology not further specified

bw, body weight; d, day or days; F, female; M, male; MC, monohybrid cross; mo, month or months; NR, not reported; d, day; wk, week or weeks

incidence of hepatocellular tumours (benign and/or malignant) in both sexes ([Vesselinovitch et al., 1975, 1979](#); [Miyakawa & Yoshida, 1980](#); [Vesselinovitch, 1983](#); [Nelson et al., 1982](#); [Littlefield et al., 1983, 1984](#)). Oral administration of benzidine caused a markedly increased incidence of mammary carcinomas in female rats ([Griswold et al., 1968](#)). Subcutaneous administration of benzidine or its sulfate to male and female rats produced a high incidence of external auditory canal carcinomas ([Spitz et al., 1950](#)), while subcutaneous administration of benzidine to frogs caused an increase in tumours of the liver and haematopoietic system ([Khudoley, 1977](#)). The intraperitoneal administration of benzidine to female rats resulted in an increase in the incidence of mammary gland adenocarcinomas and combined benign and malignant Zymbal gland tumours ([Morton et al., 1981](#)). Other studies were found to be inadequate for evaluation.

#### 4. Other Relevant Data

A general section on “Aromatic amines: metabolism, genotoxicity, and cancer susceptibility” appears as Section 4.1 in the *Monograph on 4-aminobiphenyl* in this Volume.

Benzidine, *N*-acetylbenzidine, and *N,N'*-diacetylbenzidine have been detected in the urine of workers exposed to benzidine. The predominant DNA adduct identified in exfoliated bladder cells was *N'*-(deoxyguanosin-8-yl)-*N*-acetylbenzidine ([Rothman et al., 1996a](#)). Thus, *N*-monoacetylation of benzidine does not interfere with the formation of a DNA-reactive intermediate, and may occur before the cytochrome P450-catalysed formation of *N'*-hydroxy-*N*-acetylbenzidine. In contrast, *N,N'*-diacetylation may be a detoxification pathway. *N*-Acetylbenzidine may be *N*-glucuronidated or *N*-hydroxylated in the liver. In the bladder, the *N'*-hydroxyl-*N*-acetylbenzidine

or *N'*-acetoxy-*N*-acetylbenzidine formed by NAT-mediated *O*-acetylation may react with DNA to form covalent adducts. NAT1 is more efficient than NAT2 in catalysing the *N*-acetylation of benzidine or of *N*-acetylbenzidine ([Zenser et al., 1996](#)). At low exposure levels, *N*-acetylation of benzidine is favoured over that of *N*-acetylbenzidine. Human neutrophils can also form *N'*-(deoxyguanosin-8-yl)-*N*-acetylbenzidine from *N*-acetylbenzidine by a reaction catalysed by myeloperoxidase ([Lakshmi et al., 2000](#)). *N'*-hydroxy-*N*-acetylbenzidine may also be formed through peroxidative activation by prostaglandin H synthase ([Zenser et al., 1999a, b](#)). Levels of benzidine-related DNA adducts in exfoliated urothelial cells among exposed workers were not affected by acetylator phenotype ([Rothman et al., 1996a](#)), or *GSTM1* genotype ([Rothman et al., 1996b](#)).

For several benzidine-based azo dyes, both metabolism and molecular changes identical to those of benzidine have been observed. Metabolic conversion of Direct Black 38, Direct Blue 6, and Direct Brown 95 to benzidine has been observed in the Rhesus monkey ([Rinde & Troll, 1975](#)). Azoreductase activity in intestinal bacteria and in the liver catalyses the formation of benzidine from benzidine-based dyes ([Cerniglia et al., 1982](#); [Bos et al., 1986](#)).

Benzidine is a multiorgan carcinogen in experimental animals; it induces bladder tumours in dogs, liver tumours in mice and hamsters, and mammary gland tumours in rats. In the presence of a liver-derived metabolic activation system – which in some cases leads to reductive metabolism followed by oxidative metabolism – benzidine and benzidine-based dyes (e.g. Direct Black 38, CI Acid Red 114, CI Direct Blue 15, and CI Pigment Red) were mutagenic in several strains of *S. typhimurium*. Benzidine, *N*-acetylbenzidine, and *N,N'*-diacetylbenzidine have been measured in the urine of workers exposed to Direct Black 38, and benzidine- or 4-ABP-related haemoglobin adducts have been



measured in blood ([Dewan et al., 1988](#); [Beyerbach et al., 2006](#)). Significant increases in the incidence of chromosomal aberrations in peripheral lymphocytes have been observed in workers exposed to benzidine or benzidine-based dyes ([Mirkova & Lalchev, 1990](#)). In workers exposed to benzidine, the accumulation of mutant p53 protein increased with increasing exposures ([Xiang et al., 2007](#)). Similarly, benzidine induced DNA lesions in *TP53* in the bladder, liver, and lung of exposed rats ([Wu & Heng, 2006](#)), increased the frequency of micronucleated bone-marrow cells and induced unscheduled DNA synthesis in mice, and increased DNA strand-breaks in the liver of exposed rats.

Based on bio-monitoring studies in workers, animal carcinogenicity data and genotoxicity data, it is reasonable to use the same carcinogenic hazard classification for benzidine-based dyes that are metabolized to benzidine as for benzidine.

## 5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of benzidine. Benzidine causes cancer of the urinary bladder.

There is *sufficient evidence* in experimental animals for the carcinogenicity of benzidine.

There is strong mechanistic evidence indicating that the carcinogenicity of benzidine in humans operates by a genotoxic mechanism of action that involves metabolic activation, formation of DNA adducts, and induction of mutagenic and clastogenic effects. Metabolic activation to DNA-reactive intermediates occurs by multiple pathways including *N*-oxidation in the liver, *O*-acetylation in the bladder, and peroxidative activation in the mammary gland and other organs.

Benzidine is *carcinogenic to humans (Group 1)*.

## References

- Ahlström LH, Sparr Eskilsson C, Björklund E (2005). Determination of banned azo dyes in consumer goods. *Trends Analyt Chem*, 24: 49–56. doi:10.1016/j.trac.2004.09.004
- ATSDR (2001). *Toxicological Profile for Benzidine*. Atlanta, GA: Agency for Toxic Substances and Disease Registry, 242 pp.
- Beyerbach A, Rothman N, Bhatnagar VK et al. (2006). Hemoglobin adducts in workers exposed to benzidine and azo dyes. *Carcinogenesis*, 27: 1600–1606. doi:10.1093/carcin/bgi362 PMID:16497705
- Bonser GM, Clayson DB, Jull JW (1956). The induction of tumours of the subcutaneous tissues, liver and intestine in the mouse by certain dye-stuffs and their intermediates. *Br J Cancer*, 10: 653–667. doi:10.1038/bjc.1956.79 PMID:13426377
- Bos RP, van der Krieken W, Smeijsters L et al. (1986). Internal exposure of rats to benzidine derived from orally administered benzidine-based dyes after intestinal azo reduction. *Toxicology*, 40: 207–213. doi:10.1016/0300-483X(86)90080-6 PMID:3726894
- CAREX (1999). Carex: industry specific estimates – Summary. Available at [http://www.ttl.fi/en/chemical\\_safety/carex/Documents/5\\_exposures\\_by\\_agent\\_and\\_industry.pdf](http://www.ttl.fi/en/chemical_safety/carex/Documents/5_exposures_by_agent_and_industry.pdf)
- Carreón T, LeMasters GK, Ruder AM, Schulte PA (2006a). The genetic and environmental factors involved in benzidine metabolism and bladder carcinogenesis in exposed workers. *Front Biosci*, 11: 2889–2902. doi:10.2741/2017 PMID:16720360
- Carreón T, Ruder AM, Schulte PA et al. (2006b). NAT2 slow acetylation and bladder cancer in workers exposed to benzidine. *Int J Cancer*, 118: 161–168. doi:10.1002/ijc.21308
- Case RA, Hosker ME, McDONALD DB, Pearson JT (1954). Tumours of the urinary bladder in workmen engaged in the manufacture and use of certain dyestuff intermediates in the British chemical industry. I. The role of aniline, benzidine, alpha-naphthylamine, and beta-naphthylamine. *Br J Ind Med*, 11: 75–104. PMID:13149741
- Cerniglia CE, Freeman JP, Franklin W, Pack LD (1982). Metabolism of benzidine and benzidine-congener based dyes by human, monkey and rat intestinal bacteria. *Biochemical and Biophysical Research Communications*, 107: 1224–1229. doi:10.1016/S0006-291X(82)80128-9 PMID:6814437
- Dewan A, Jani JP, Patel JS et al. (1988). Benzidine and its acetylated metabolites in the urine of workers exposed to Direct Black 38. *Arch Environ Health*, 43: 269–272. PMID:3415352
- ETAD (2008). The restrictions on the marketing and use of azo colourants according to the European legislation

- following the Directive 2002/61/EC (19<sup>th</sup> Amendment of Council Directive 76/769/EEC) (ETAD Information Notice No. 6)
- Garrigós MC, Reche F, Marín ML, Jiménez A (2002). Determination of aromatic amines formed from azo colorants in toy products. *J Chromatogr A*, 976: 309–317. doi:10.1016/S0021-9673(02)01162-7 PMID:12462623
- Griswold DP Jr, Casey AE, Weisburger EK, Weisburger JH (1968). The carcinogenicity of multiple intragastric doses of aromatic and heterocyclic nitro or amino derivatives in young female sprague-dawley rats. *Cancer Res*, 28: 924–933. PMID:5652305
- Hu J, Mao Y, White K (2002). Renal cell carcinoma and occupational exposure to chemicals in Canada. *Occup Med (Lond)*, 52: 157–164. doi:10.1093/occmed/52.3.157 PMID:12063361
- IARC (1972). Some inorganic substances, chlorinated hydrocarbons, aromatic amines, N-nitroso compounds and natural products. *IARC Monogr Eval Carcinog Risk Chem Man*, 1: 1–184.
- IARC (1982). Some industrial chemicals and dyestuffs. *IARC Monogr Eval Carcinog Risk Chem Hum*, 29: 1–398. PMID:6957379
- IARC (1987). Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42. *IARC Monogr Eval Carcinog Risks Hum Suppl*, 7: 1–440. PMID:3482203
- IARC (2010). Some aromatic amines, organic dyes, and related exposures. *IARC Monogr Eval Carcinog Risks Hum*, 99: 1–678. PMID:21528837.
- Kauppinen T, Toikkanen J, Pedersen D *et al.* (2000). Occupational exposure to carcinogens in the European Union. *Occup Environ Med*, 57: 10–18. doi:10.1136/oem.57.1.10 PMID:10711264
- Khudoley VV (1977). Tumor induction by carcinogenic agents in anuran amphibian *Rana temporaria*. *Arch Geschwulstforsch*, 47: 385–399. PMID:303891
- Lakshmi VM, Hsu FF, Davis BB, Zenser TV (2000). N-Acetylbenzidine-DNA adduct formation by phorbol 12-myristate-stimulated human polymorphonuclear neutrophils. *Chemical Research in Toxicology*, 13: 785–792. doi:10.1021/tx0000320 PMID:10956067
- Lancaster FE & Lawrence JF (1999). Determination of benzidine in the food colours tartrazine and sunset yellow FCF, by reduction and derivatization followed by high-performance liquid chromatography. [abstract]*Food Addit Contam*, 16: 381–390. doi:10.1080/026520399283867 PMID:10755129
- Lide DR, editor (2008). *CRC Handbook of Chemistry and Physics*, 89<sup>th</sup> ed. Boca Raton, FL: CRC Press, pp. 3–36.
- Littlefield NA, Nelson CJ, Frith CH (1983). Benzidine dihydrochloride: toxicological assessment in mice during chronic exposures. *J Toxicol Environ Health*, 12: 671–685. doi:10.1080/15287398309530459 PMID:6366243
- Littlefield NA, Nelson CJ, Gaylor DW (1984). Benzidine dihydrochloride: risk assessment. *Fundam Appl Toxicol*, 4: 69–80. doi:10.1016/0272-0590(84)90220-3 PMID:6363187
- Meigs JW, Marrett LD, Ulrich FU, Flannery JT (1986). Bladder tumor incidence among workers exposed to benzidine: a thirty-year follow-up. *J Natl Cancer Inst*, 76: 1–8. PMID:3455732
- Mirkova ET & Lalchev SG (1990). The genetic toxicity of the human carcinogens benzidine and benzidine-based dyes: chromosomal analysis in exposed workers. *Prog Clin Biol Res*, 340C: 397–405. PMID:2381938
- Miyakawa M & Yoshida O (1980). Protective effects of DL-tryptophan on benzidine-induced hepatic tumor in mice. *Gann*, 71: 265–268. PMID:7053208
- Morton KC, Wang CY, Garner CD, Shirai T (1981). Carcinogenicity of benzidine, N,N'-diacetylbenzidine, and N-hydroxy-N,N'-diacetylbenzidine for female CD rats. *Carcinogenesis*, 2: 747–752. doi:10.1093/carcin/2.8.747 PMID:7285281
- Nelson CJ, Baetcke KP, Frith CH *et al.* (1982). The influence of sex, dose, time, and cross on neoplasia in mice given benzidine dihydrochloride. *Toxicol Appl Pharmacol*, 64: 171–186. doi:10.1016/0041-008X(82)90214-9 PMID:7123548
- NIOSH (1984). National Occupational Exposure Survey, 1981–1983. Available at <http://www.cdc.gov/noes/noes3/empl0003.html>
- NTP (2005). NTP 11th Report on Carcinogens. *Rep Carcinog*, 11: 1–A32. PMID:19826456
- O'Neil MJ, editor (2006). *The Merck Index*, 14<sup>th</sup> ed. Whitehouse Station, NJ: Merck & Co. Inc., p. 179.
- Piolatto G, Negri E, La Vecchia C *et al.* (1991). Bladder cancer mortality of workers exposed to aromatic amines: an updated analysis. *Br J Cancer*, 63: 457–459. doi:10.1038/bjc.1991.106 PMID:2003988
- Rinde E & Troll W (1975). Metabolic reduction of benzidine azo dyes to benzidine in the rhesus monkey. *J Natl Cancer Inst*, 55: 181–182. PMID:808635
- Rothman N, Bhatnagar VK, Hayes RB *et al.* (1996a). The impact of interindividual variation in NAT2 activity on benzidine urinary metabolites and urothelial DNA adducts in exposed workers. *Proceedings of the National Academy of Sciences of the United States of America*, 93: 5084–5089. doi:10.1073/pnas.93.10.5084 PMID:8643532
- Rothman N, Hayes RB, Zenser TV *et al.* (1996b). The glutathione S-transferase M1 (GSTM1) null genotype and benzidine-associated bladder cancer, urine mutagenicity, and exfoliated urothelial cell DNA adducts. *Cancer Epidemiology, Biomarkers & Prevention*, 5: 979–983. PMID: 8959320.
- Spitz S, Maguigan WH, Dobriner K (1950). The carcinogenic action of benzidine. *Cancer*, 3: 789–804. doi:10.1002/1097-0142(1950)3:5<789::AID-CNCR2820030505>3.0.CO;2-U PMID:14772711

- Sun LD & Deng XM (1980). An epidemiological survey of bladder carcinoma in chemical dye industry (author's transl) *Zhonghua Wai Ke Za Zhi*, 18: 491–493. PMID:7238196
- Vesselinovitch SD (1983). Perinatal hepatocarcinogenesis. *Biol Res Pregnancy Perinatol*, 4: 22–25. PMID:6303459
- Vesselinovitch SD, Rao KV, Mihailovich N (1979). Neoplastic response of mouse tissues during perinatal age periods and its significance in chemical carcinogenesis. *Natl Cancer Inst Monogr*, 51239–250. PMID:384263
- Vesselinovitch SD, Rao KVN, Mihailovich N (1975). Factors modulating benzidine carcinogenicity bioassay. *Cancer Res*, 35: 2814–2819. PMID:1157051
- Vigliani EC & Barsotti M (1962). Environmental tumors of the bladder in some Italian dye-stuff factories. *Acta Unio Int Contra Cancrum*, 18: 669–675. PMID:13997151
- Wu Q & Heng ZC (2006). Study of rat's p53 gene damage and organ specificity induced by benzidine *Sichuan Da Xue Xue Bao Yi Xue Ban*, 37: 33–34, 39. PMID:16468636
- Wu W (1988). Occupational cancer epidemiology in the People's Republic of China. *J Occup Med*, 30: 968–974. doi:10.1097/00043764-198812000-00017 PMID:3068337
- Xiang CQ, Shen CL, Wu ZR *et al.* (2007). Detection of mutant p53 protein in workers occupationally exposed to benzidine. *Journal of Occupational Health*, 49: 279–284. doi:10.1539/joh.49.279 PMID:17690521
- Zenser TV, Lakshmi VM, Hsu FF *et al.* (1999a). Metabolism of N-acetylbenzidine and initiation of bladder cancer. *Mutation Research*, 506–507: 29–40. PMID: 12351142.
- Zenser TV, Lakshmi VM, Hsu FF, Davis BB (1999b). Peroxygenase metabolism of N-acetylbenzidine by prostaglandin H synthase. Formation of an N-hydroxylamine. *The Journal of Biological Chemistry*, 274: 14850–14856. doi:10.1074/jbc.274.21.14850 PMID:10329684
- Zenser TV, Lakshmi VM, Rustan TD *et al.* (1996). Human N-acetylation of benzidine: role of NAT1 and NAT2. *Cancer Res*, 56: 3941–3947. PMID:8752161

