

# DYES METABOLIZED TO BENZIDINE

Dyes metabolized to benzidine (benzidine-based dyes) were considered by a previous IARC Working Group in 2008 ([IARC, 2010](#)). Three azo dyes metabolized to benzidine, Direct Black 38, Direct Blue 6, and Direct Brown 95 were considered by IARC Working Groups in 1982, 1987, and 2008 ([IARC, 1982, 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

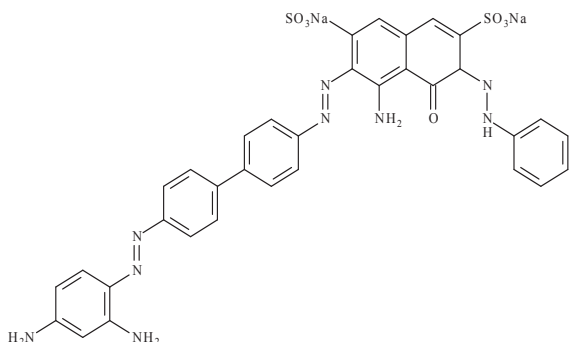
### 1.1 Identification of the agents

#### 1.1.1 Direct Black 38

*Chem. Abstr. Serv. Reg. No.:* 1937-37-7

*Chem. Abstr. Serv. Name:* 4-amino-3-[2-[4'-[2-(2,4-diaminophenyl)diazenyl][1,1'-biphenyl]-4-yl]diazenyl]-5-hydroxy-6-(2-phenyldiazenyl)-2,7-naphthalenedisulfonic acid, sodium salt (1:2)

*Synonyms:* C.I. 30235; C.I. Direct Black 38, disodium salt



$C_{34}H_{25}N_9Na_2O_7S_2$

Relative molecular mass: 781.72

*Description:* Black powder

*Solubility:* Soluble in water; moderately soluble in ethanol and ethylene glycol mono-ethyl ether; insoluble in other organic solvents ([IARC, 2010](#))

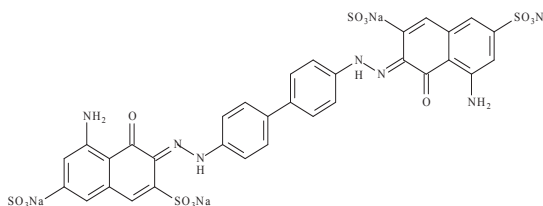
#### 1.1.2 Direct Blue 6

*Chem. Abstr. Serv. Reg. No.:* 2602-46-2

*Chem. Abstr. Serv. Name:*

3,3'-[[1,1'-biphenyl]-4,4'-diylbis(2,1-diazenediyl)]bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid], sodium salt (1:4)

*Synonyms:* C.I. 22610; C.I. Direct Blue 6; C.I. Direct Blue 6, tetra-sodium salt



$C_{32}H_{20}N_6Na_4O_{14}S_4$

Relative molecular mass: 932.74

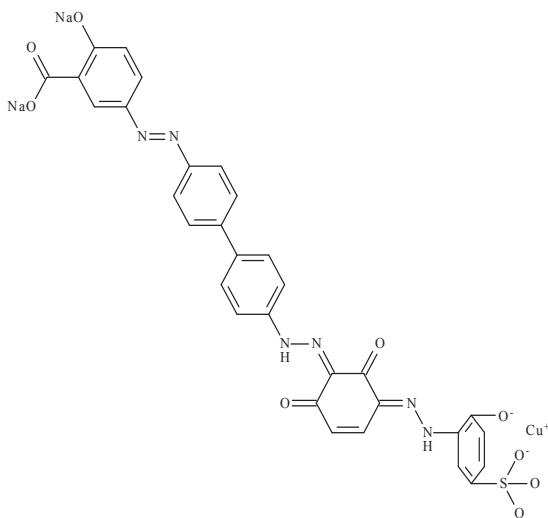
*Description:* Blue powder

*Solubility:* Soluble in water; moderately soluble in ethanol and ethylene glycol mono-ethyl ether; insoluble in other organic solvents ([IARC, 2010](#))

### 1.1.3 Direct Brown 95

*Chem. Abstr. Serv. Reg. No.:* 16071-86-6  
*Chem. Abstr. Serv. Name:* [2-hydroxy-5-[2-[4'-[2-[2-(hydroxy-κO)-6-hydroxy-3-[2-[2-(hydroxy-κO)-5-sulfophenyl]diazenyl-κN1]phenyl]diazenyl][1,1'-biphenyl]-4-yl]diazenyl]benzoato(4-)-cuprate(2-), sodium (1:2)

*Synonyms:* C.I. 30145; C.I. Direct Brown 95



$C_{31}H_{18}CuN_6Na_2O_9S$

Relative molecular mass: 760.10

*Description:* Reddish-brown powder

*Solubility:* Soluble in water; moderately soluble in ethanol; insoluble in acetone

([IARC, 2010](#))

## 1.2 Uses

In general, benzidine-based dyes were used primarily to colour textiles, leather, and paper products and also in the petroleum, rubber, plastics, wood, soap, fur, and hair-dye industries. More than 300 benzidine-based dyes are listed in the Colour Index, including 18 that were commercially available in the United States of America (USA). Access to these dyes for home use is no longer permitted in the USA, but some – in particular direct browns, greens, and blacks – were available as consumer products in the 1970s

([IARC, 2010](#)). Direct Black 38, Direct Blue 6, and Direct Brown 95 were used on textiles (such as cotton, silk, wool, nylon, and acetate) and on leather. In addition, Direct Black 38 and Direct Blue 6 were used in aqueous printing inks, in hair dyes and as biological stains; Direct Black 38 and Direct Brown 95 were used in plastics; Direct Blue 6 and Direct Brown 95 were used in paper; and, Direct Black 38 was used in wood stains and wood flour ([Society of Dyers and Colourists, 1971](#); [NCI, 1978](#)).

By the mid-1970s, most manufacturers in the USA started phasing out the use of benzidine-based dyes and replacing them with other types of dyes ([NIOSH, 1980](#)). 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 ppm). In 2002, the European Union published a Directive (76/769/EEC) that expanded the coverage to compounds that come in contact with the oral cavity, and added two amines to the list ([Ahlström et al., 2005](#); [ETAD, 2008](#)).

## 1.3 Human exposure

### 1.3.1 Occupational exposure

Occupational exposure to benzidine-based dyes can occur during the production and use of these substances. The primary routes of potential exposure to benzidine-based dyes are inhalation and accidental ingestion; dermal absorption can also occur. The potential for exposure has declined since the late 1970s, as benzidine-based dyes were removed from both industrial and consumer markets and replaced with other types of dye. Since 1980, use of mixtures containing benzidine at concentrations of 0.1% or more is

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

Industry, occupational activity	
Manufacture of textiles	650
Manufacture of leather and products of leather or of its substitutes	220
Manufacture of furniture and fixtures, except primarily of metal	380
Manufacture of plastic products not elsewhere classified	1470
Iron and steel basic industries	10
Manufacture of fabricated metal products, except machinery and equipment	20
Manufacture of machinery except electrical	640
Manufacture of instruments, and of photographic and optical goods	50
Other manufacturing industries	120
Research and scientific institutes	9280
Medical, dental, other health and veterinary services	1090
TOTAL	13920

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

permitted only in closed systems; all workers must observe special precautions to reduce exposure, and strict procedures must be followed to transport such materials ([IARC, 1982](#)).

Exposure studies in workers involved in manufacture and use of benzidine-based dyes generally were designed to measure benzidine rather than the benzidine-based dyes. Studies that reported airborne and urinary concentrations and results of dermal wipes of benzidine in the benzidine-based dye industry have been reviewed ([IARC, 2010](#)).

Estimates of numbers of workers potentially exposed to benzidine-based dyes 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 that 13920 workers were exposed to benzidine-based dyes in the European Union ([CAREX, 1999](#); [Table 1.1](#)).

From the US National Occupational Hazard Survey (1972–74) it was estimated that 79200

workers in 63 occupations (primarily the dye manufacturing, textile dyeing, printing, paper, and leather industries) were potentially exposed to benzidine-based dyes ([NIOSH, 1980](#)). A decade later, estimates from another US National Occupational Exposure Survey (1981–83) indicated that about 33900 workers were potentially exposed to one or more of 13 benzidine-based dyes. Although no current estimate of occupational exposure to this type of dye is available, the number of potentially exposed workers is expected to be much lower than in the past ([NTP, 2005](#)).

### 1.3.2 Non-occupational exposure

Benzidine-based dyes can contain various amounts of benzidine as a contaminant. The general population can be exposed to benzidine-based dyes when in contact with consumer goods containing such dyes, e.g. 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](#)).

Benzidine and benzidine-based dyes have been detected in waste effluents from plants manufacturing or using these dyes ([IARC, 2010](#)).

## 2. Cancer in Humans

Benzidine-based dyes (i.e. dyes metabolized to benzidine) were most recently reviewed in *IARC Monograph* Volume 99 ([IARC, 2010](#)).

### 2.1 Cohort Studies

Three azo dyes metabolized to benzidine, Direct Black 38, Direct Blue 6 and Direct Brown 95, were evaluated in previous *IARC Monographs* ([IARC, 1982, 1987, 2010](#)). The most recent evaluation ([IARC, 2010](#)) stated that there was *inadequate evidence* in humans for the carcinogenicity of dyes metabolized to benzidine. [Stern et al. \(1987\)](#) studied 9365 production workers (7085 men, 2280 women) employed at two leather tanneries where detectable quantities of benzidine were identified in bulk dyes (probably derived from the dyes). Excesses of cancer of the urinary bladder were not observed. The same author ([Stern, 2003](#)) conducted a follow-up study with the same result (see Table 2.1, available online at <http://monographs.iarc.fr/ENG/Monographs/vol100F/100F-03-Table2.1.pdf>). [Costantini et al. \(1989\)](#) carried out a cohort-mortality study of 2926 male workers employed in tanneries between 1950 and 1981 in Italy. Known exposures in the plants included dyes metabolized to benzidine, dyes metabolized to 3,3'-dimethylbenzidine and dyes metabolized to 3,3'-dimethoxybenzidine. No significant excesses of cancer of the urinary bladder were found (5 deaths, SMR 1.5, 95%CI: 0.5–3.5). [You et al. \(1990\)](#) observed only one case of cancer of the urinary bladder in 1210 workers (1060 men, 150 women) from 17 knitting factories, 10 stocking factories, nine silk printing and dyeing

factories and seven printing and dyeing factories in China. Fifteen types of benzidine-derived dye had been used in these 43 factories, Direct Black 38 being the most common. [Montanaro et al. \(1997\)](#) reported on mortality in a cohort of 1224 workers employed at a chrome tannery in Italy between 1955 and 1988 who were exposed to azo dyes metabolized to benzidine and other chemicals in the tanning process. An excess risk for cancer of the urinary bladder was observed (10 deaths, SMR 2.4, 95%CI: 1.2–4.5).

### 2.2 Case-control studies

[Yoshida et al. \(1971\)](#) reported an excess risk for cancer of the urinary bladder (OR, 6.8,  $P = 0.002$ ) in male workers in the silk-dyeing industry in Japan, in a case-control study of 200 bladder-cancer cases and 148 controls. At least seven of the 17 patients with bladder cancer worked as kimono painters, reportedly using benzidine-based dyes. [Myslak et al. \(1991\)](#) found an excess risk for cancer of the urinary bladder among painters in Germany (OR, 2.9, 95%CI: 1.3–6.6). Benzidine-based dyes were manufactured in that country before 1950 and painters were reported to prepare the paints themselves. These studies do not provide data specifically on dyes metabolized to benzidine.

### 2.3 Synthesis

There is a lack of clear and consistent evidence from epidemiological studies that dyes metabolized to benzidine cause cancer in humans.

## 3. Cancer in Experimental Animals

Studies on the carcinogenicity of dyes metabolized to benzidine in mice and rats exposed by the oral route or through bladder implantation have been reviewed ([IARC, 1987, 2010](#)). There

**Table 3.1 Carcinogenicity studies of dyes metabolized to benzidine in experimental animals**

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
<b>Direct Black 38</b>				
Mouse, ICR (M, F) 60 wk <a href="#">Asada et al. (1981)</a>	Drinking-water Groups of 60 mice were given drinking-water containing 3 g/L Direct Black 38. Surviving animals were sacrificed 60 wk after the start of the study. A group of 20 mice served as untreated controls.	Hepatocellular carcinoma: 0/20, 46/59 Mammary gland cancers: 0/20, 20/59	[ $P < 0.0001$ ] [ $P < 0.0005$ ]	Gender distribution NR Purity NR
Rat, F344 (M, F) 13 wk <a href="#">NTP (1978)</a> , <a href="#">Robens et al. (1980)</a>	Feed Groups of 10 male and 10 female rats were fed 0, 190, 375, 750, 1500, or 3000 ppm Direct Black 38 (87% pure, containing ~7% water, ~8% NaCl, no benzidine detected) in the diet for 13 wk and then sacrificed.	Hepatocellular carcinoma or neoplastic nodules: M-0/10, 0/10, 0/10, 0/10, 9/9* (hepatocellular carcinoma, 4/9**), 0/9; F-0/10, 0/10, 0/10, 0/10, 5/10** (only neoplastic nodules), 0/8	* $P < 0.001$ Fisher exact test ** $P = 0.01-0.05$ Fisher exact test	Study is of short duration with small number of animals. High mortality in highest dose groups
<b>Direct Blue 6</b>				
Rat, F344 (M, F) 13 wk <a href="#">NTP (1978)</a> , <a href="#">Robens et al. (1980)</a>	Feed Groups of 10 male and 10 female rats were fed 0, 190, 375, 750, 1500, or 3000 ppm Direct Blue 6 (60% pure, containing ~9% water, ~21% NaCl, no benzidine detected) in the diet for 13 wk and then sacrificed.	Hepatocellular carcinoma or neoplastic nodules: M-0/10, 0/10, 0/10, 0/10, 8/10* (hepatocellular carcinoma, 2/10), 1/9 F-0/10, 0/10, 0/10, 0/10, 0/10, 7/9** (hepatocellular carcinoma, 4/9***)	* $P < 0.001$ Fisher exact test ** $P = 0.001-0.009$ Fisher exact test *** $P = 0.01-0.05$	Study is of short duration with small number of animals. High mortality in highest dose groups
Mouse, dd (F) 40 wk <a href="#">Niitsu (1973)</a>	Bladder implantation Two groups of 50 female mice received either a paraffin wax pellet (20 mg) containing 10% Direct Blue BB or a wax pellet alone implanted in the bladder, and were kept for up to 40 wk.	Bladder carcinomas: 1/36 (2.7%), 3/21(14.2%)	[NS, Fisher exact test]	Study duration was short. "Direct Blue BB" is a synonym for Direct Black 38. Purity unspecified. Age not specified (weight, 20 g).

**Table 3.1 (continued)**

Species, strain (sex) Duration Reference	Route Dosing regimen, Animals/group at start	Incidence of tumours	Significance	Comments
<b>Direct Brown 95</b> Rat, F344 (M, F) 13 wk <a href="#">NTP (1978)</a> , <a href="#">Robens et al. (1980)</a>	Feed Groups of 10 male and 10 female rats were fed 0, 190, 375, 750, 1500, or 3000 ppm Direct Brown 95 (72% pure, containing ~5% water, ~15% NaCl, no benzidine detected) in the diet for 13 wk, and then sacrificed.	Hepatocellular carcinoma or neoplastic nodules: M–0/10, 0/10, 0/10, 0/9, 0/9, 0/9; F–0/10, 0/10, 0/10, 0/10, 5/8* (hepatocellular carcinoma, 1/8), 0/8	* $P = 0.001 - 0.009$ Fisher exact test	Study is of short duration with small number of animals. High mortality in highest dose groups.

F, female; M, male; NR, not reported; NS, not significant; wk, week or weeks

have been no additional carcinogenicity studies in animals reported since those reviews. Results of adequately conducted carcinogenicity studies are summarized in [Table 3.1](#).

Three dyes that would be metabolized to benzidine, Direct Black 38, Direct Blue 6 and Direct Brown 95, were tested for carcinogenicity by oral administration (feed or drinking-water) or bladder implantation. Direct Black 38 was adequately tested in one drinking-water study in mice and in one feeding study in mice and rats. Following its oral administration to mice in the drinking-water for 60 weeks, Direct Black 38 significantly increased the incidence of hepatocellular carcinomas and mammary gland cancers ([Asada et al., 1981](#)). Oral administration of Direct Black 38 to male and female rats in the diet for only 13 weeks caused a significant increase of hepatocellular carcinomas in males and of liver neoplastic nodules in both sexes ([NTP, 1978](#); [Robens et al., 1980](#)). Direct Blue 6 was adequately tested in one feeding study in mice and rats and one bladder-implantation study in mice. Oral administration of Direct Blue 6 to male and female rats in the diet for only 13 weeks caused a significant increase in hepatocellular carcinomas in females and of hepatocellular carcinomas and liver neoplastic nodules combined in both sexes ([NTP, 1978](#); [Robens et al., 1980](#)). The bladder-implantation study in mice showed a slight increase in transitional cell carcinoma of the urinary bladder ([Niitsu, 1973](#)). Direct Brown 95 was adequately tested in a feeding study in mice and rats. Oral administration of Direct Brown 95 to male and female rats in the diet for only 13 weeks caused a significant increase in hepatocellular carcinomas and liver neoplastic nodules combined in females ([NTP, 1978](#); [Robens et al., 1980](#)).

## 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.

For details on benzidine and benzidine-based dyes, see Section 4 of the *Monograph* on Benzidine in this volume.

## 5. Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of dyes metabolized to benzidine.

There is *sufficient evidence* in experimental animals for the carcinogenicity of Direct Black 38, Direct Blue 6, and Direct Brown 95.

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

There is strong mechanistic evidence indicating that benzidine-based dyes are converted by azoreduction to benzidine in humans and in experimental animals and, consequently, produce DNA adducts and genotoxic effects similar to those of benzidine.

Dyes metabolized to benzidine are *carcinogenic to humans (Group 1)*.

In making the overall evaluation, the Working Group considered the following:

- there is *sufficient evidence* in humans and in experimental animals for the carcinogenicity of benzidine.
- the metabolism of benzidine-based dyes results in the release of free benzidine and the induction of chromosomal aberrations in humans, and in all experimental animal species studied.

## References

- Ahlström LH, Sparr Eskilsson C, Björklund E (2005). Determination of banned azo dyes in consumer goods. *Trends in Analytical Chemistry*, 24: 49–56. doi:10.1016/j.trac.2004.09.004
- Asada I, Matsumoto Y, Tobe T *et al.* (1981). Induction of hepatoma in mice by direct deep Black-Extra (DDB-EX) and occurrence of serum AFP. *Nippon Geka Hokan*, 50: 45–55. PMID:6169317
- 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)
- Costantini AS, Paci E, Miligi L *et al.* (1989). Cancer mortality among workers in the Tuscan tanning industry. *Br J Ind Med*, 46: 384–388. PMID:2818971
- 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. *Journal of Chromatography A*, 976: 309–317. doi:10.1016/S0021-9673(02)01162-7 PMID:12462623
- 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: 1–658. PMID:21528837
- Kauppinen T, Toikkanen J, Pedersen D *et al.* (2000). Occupational exposure to carcinogens in the European Union. *Occupational and Environmental Medicine*, 57: 10–18. doi:10.1136/oem.57.1.10 PMID:10711264
- 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 Additives and Contaminants*, 16: 381–390. doi:10.1080/026520399283867 PMID:10755129
- Montanaro F, Ceppi M, Demers PA *et al.* (1997). Mortality in a cohort of tannery workers. *Occupational and Environmental Medicine*, 54: 588–591. doi:10.1136/oem.54.8.588 PMID:9326162
- Myslak ZW, Bolt HM, Brockmann W (1991). Tumors of the urinary bladder in painters: a case-control study. *American Journal of Industrial Medicine*, 19: 705–713. doi:10.1002/ajim.4700190604 PMID:1882850
- NCI (1978). *Thirteen-Week Subchronic Toxicity Studies of Direct Blue 6, Direct Black 38 and Direct Brown 95 Dyes*. Technical Report Series No 108. DHEW (NIH) Publication No. 78–1358. Bethesda, MD: National Institutes of Health.
- Niitsu K (1973). Studies of the metabolism and carcinogenicity of azo dyes used for food colors and direct dyestuffs. Part II. Studies on the metabolism and carcinogenicity of direct dyestuffs blue BB and black EX. *Tokyo Jikeikai Ika Daigaku Zasshi*, 88: 467–471.
- NIOSH (1980). *Special Occupational Hazard Review for Benzidine-Based Dyes*. NIOSH Criteria Documents. DHHS (NIOSH) Publication No. 80–109. U.S. Department of Health, Education and Welfare, Public Health Services, Center for Disease Control. 60 pp. [<http://www.cdc.gov/niosh/80-109.html>]
- NTP (1978). 13-week subchronic toxicity studies of direct blue 6, direct black 38, and direct brown 95 dyes. *Natl Cancer Inst Carcinog Tech Rep Ser*, 108: 1–117. PMID:12799683
- NTP (2005). NTP 11th Report on Carcinogens. *Rep Carcinog*, 11: 1–A32. PMID:19826456
- Robens JF, Dill GS, Ward JM *et al.* (1980). Thirteen-week subchronic toxicity studies of Direct Blue 6, Direct Black 38, and Direct Brown 95 dyes. *Toxicology and Applied Pharmacology*, 54: 431–442. doi:10.1016/0041-008X(80)90170-2 PMID:7394798
- Society of Dyers and Colourists (1971). *Colour Index*, 3<sup>rd</sup> ed., Vol. 4, Bradford, UK: Lund Humphries, p. 4742
- Stern FB (2003). Mortality among chrome leather tannery workers: an update. *Am J Ind Med*, 44: 197–206. PMID:12874853
- Stern FB, Beaumont JJ, Halperin WE *et al.* (1987). Mortality of chrome leather tannery workers and chemical exposures in tanneries. *Scand J Work Environ Health*, 13: 108–117. PMID:3602964
- Yoshida O, Harada T, Miyagawa M *et al.* (1971). Bladder cancer in workers of the dyeing industry - epidemiological survey focusing on Kyoto Prefecture *Igaku no Ayumi*, 79: 421–422.
- You XY, Chen JG, Hu YN (1990). Studies on the relation between bladder cancer and benzidine or its derived dyes in Shanghai. *Br J Ind Med*, 47: 544–552. PMID:2393635