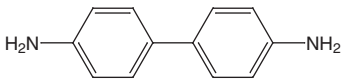
Benzidine and Dyes Metabolized to Benzidine

Introduction

Benzidine was listed for the first time in the *First Annual Report on Carcinogens* (1980), and dyes metabolized to benzidine were all listed in the *Ninth Report on Carcinogens* (2000). The profiles for benzidine and dyes metabolized to benzidine, which are listed (separately) as *known to be human carcinogens*, follow this introduction.

Benzidine
CAS No. 92-87-5

Known to be a human carcinogen
First Listed in the *First Annual Report on Carcinogens* (1980)



Carcinogenicity

Benzidine is *known to be a human carcinogen* based on sufficient evidence of carcinogenicity in humans. Numerous epidemiological studies (case reports and cohort studies) of workers in various geographical locations have reported a strong association between occupational exposure to benzidine and bladder cancer. Moreover, epidemiological data suggest that bladder cancer incidence has decreased since measures to limit benzidine exposure were instituted. A few studies have evaluated exposure to benzidine alone; however, in many studies, workers were co-exposed to other chemicals. Some studies have suggested that the risk of bladder cancer increases with increasing length of exposure to benzidine (IARC 1982, 1987). Since benzidine was reviewed for listing in the *First Annual Report on Carcinogens* and by the International Agency for Research on Cancer (IARC), some but not all studies have reported an association between benzidine exposure and cancer at other tissue sites (i.e., liver, kidney, central nervous system, oral cavity, larynx, esophagus, bile duct, gallbladder, stomach, and pancreas); the evidence for an association with benzidine is more limited for these cancers than for bladder cancer (as reviewed by Choudhary 1996).

There is sufficient evidence for the carcinogenicity of benzidine in experimental animals as demonstrated by studies showing that benzidine causes cancer in rats, mice, hamsters, and dogs. When administered orally, benzidine caused mammary-gland tumors in female rats, liver tumors in mice and hamsters, and bladder tumors in dogs. When administered by subcutaneous (s.c.) or intraperitoneal (i.p.) injection, it caused Zymbal-gland tumors (s.c. or i.p. injection) and mammary-gland tumors (i.p. injection) in rats and liver tumors in mice (s.c. injection) (IARC 1982, 1987).

Additional Information Relevant to Carcinogenicity

Benzidine is metabolized by cytochrome P-450 enzymes (via *N*-oxidation) to form electrophilic compounds that can covalently bind to DNA (Choudhary 1996). Benzidine caused mutations in bacteria and plants, but gave conflicting results in cultured rodent cells. It also caused many other types of genetic damage in various test systems, including yeast, cultured human and other mammalian cells, and rodents exposed *in vivo*. The damage included mitotic gene conversion (in yeast), micronucleus formation (a sign of chromosome damage or loss), DNA strand breaks, unscheduled DNA synthesis (a DNA repair response), cell transformation (a step in tumor formation), chromosomal aberrations (changes in chromosome structure or number), sister chromatid exchange, and aneuploidy (extra or missing chromosomes) (IARC 1987). Workers exposed to benzidine and or benzidine-based dyes had higher levels of chromosomal aberrations in their white bloods cells than did unexposed workers (Choudhary 1996).

Properties

Benzidine is an aromatic amine with a molecular weight of 184.2. It occurs as a grayish-yellow, white, or reddish-gray crystalline powder that darkens when exposed to air and light. It has a specific gravity of 1.25 at 20°C/4°C, a melting point of 128°C, and a boiling point of 401°C. Benzidine is slightly soluble in water, boiling alcohol, and ether and has a log octanol-water partition coefficient of 1.34. It can be made to sublime, and its vapor density is 6.36. Benzidine may burn but does not readily ignite (ATSDR 2001, HSDB 2003).

Use

Benzidine has been used for over a century as an intermediate in the production of azo dyes, sulfur dyes, fast color salts, naphthols, and other dyeing compounds (IARC 1982). 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 concerns about benzidine's potential carcinogenicity. Some dyes that may contain benzidine as an impurity still are used as stains for microscopy and similar laboratory applications (ATSDR 2001).

Production

Benzidine is no longer manufactured for commercial sale in the United States (ATSDR 2001). All large-scale production was discontinued in 1976, and only relatively small quantities remain available for use in diagnostic testing. All benzidine production must be for captive consumption (in-house use) and take place in closed systems under stringent workplace controls. Estimated U.S. benzidine production in 1983 was only 500 lb (227 kg) (possibly excluding some captive production), compared with 10 million pounds (4,500 metric tons) in 1972 (ATSDR 2001). Nine U.S. suppliers (ChemSources 2003) but no manufacturers (SRI 2003) of benzidine were identified in 2003.

Benzidine has not been imported into the United States in recent years. In 1980, the last year for which an estimate was found, 8,900 lb (4,000 kg) of benzidine was imported into the United States (ATSDR 2001). No data on exports were found.

Exposure

Because benzidine may be produced only for captive consumption, its direct release into the environment is expected to be low. However, accidental releases from closed systems potentially could result in environmental exposure through inhalation, ingestion, or dermal contact. In the past, benzidine may have been released into wastewaters and sludges. Because benzidine is moderately persistent in the environment, exposure of populations living near former benzidine or benzidine-dye manufacturing or waste-disposal sites may still be of concern. Benzidine has been identified in 28 of 1,585 hazardous waste sites proposed for inclusion on the U.S. Environmental Protection Agency (EPA) National Priorities List; however, it is not known how many sites were evaluated for benzidine. In 1990, benzidine was detected at 240 µg/L (on site) and 19 µg/L (off site) in groundwater at a hazardous waste site that was the former location of a large dye manufacturer (ATSDR 2001).

Current industrial release of benzidine to the environment is limited. The U.S. EPA's Toxics Release Inventory listed four facilities that released a total of 532 lb (241 kg) of benzidine to the environment in 2001, including 17 lb (8 kg) to the air and 300 lb (136 kg) to surface

water. Reported industrial releases of benzidine were 16 lb (7 kg) in 1993, 250 lb (113 kg) in 1994, and 2 lb (1 kg) in 1999 (TRI01 2003).

Benzidine-based dyes may still be imported into the United States, and microbial degradation of these dyes may release free benzidine into the environment (ATSDR 2001). The U.S. Food and Drug Administration limits the benzidine content in food colorants to 1 ppb; however, other impurities in synthetic coloring agents may be metabolized to benzidine after ingestion.

Before OSHA regulations were adopted to limit occupational exposure to benzidine (starting in 1974), benzidine and its derivatives were manufactured and used in open systems that permitted release of benzidine into workplace air. Air concentrations of benzidine measured in a benzidine manufacturing plant ranged from 0.007 to 17.6 mg/m^3, and levels in the urine of exposed workers ranged from 1 to 112 µg/L (ATSDR 2001). The National Occupational Exposure Survey (1981–1983) estimated that 15,554 workers, including 426 women, potentially were exposed to benzidine (NIOSH 1984). Benzidine is available in limited quantities for use as a research chemical and may be present as a trace impurity in stains used by medical or laboratory technicians. Others potentially exposed to benzidine include workers involved in its production in closed systems and workers at hazardous waste sites where benzidine is present (ATSDR 2001).

Regulations

DOT

Benzidine is considered a hazardous material and special requirements have been set for marking, labeling, and transporting this material

EPA

Clean Air Act

NESHAP: Listed as a Hazardous Air Pollutant (HAP)

Clean Water Act

Effluent Guidelines: Listed as a Toxic Pollutant

Water Quality Criteria: Based on fish/shellfish and water consumption = 0.000086 µg/L; based on fish/shellfish consumption only = 0.00020 µg/L

Comprehensive Environmental Response, Compensation, and Liability Act

Reportable Quantity (RQ) = 1 lb

Emergency Planning and Community Right-To-Know Act

Toxics Release Inventory: Listed substance subject to reporting requirements

Resource Conservation and Recovery Act

Listed Hazardous Waste: Waste codes in which listing is based wholly or partly on substance - U021

Listed as a Hazardous Constituent of Waste

FDA

The color additives, FD&C yellow no. 5, yellow no. 6, and D&C red no. 33, may contain maximum levels of benzidine that range from 1-20 ppb

The color additive, Ext. D&C yellow no. 1, is banned because there is no assurance that it will not produce benzidine from the decomposition of a subsidiary reaction product

OSHA

Potential occupational carcinogen: Engineering controls, work practices, and personal protective equipment required

Guidelines

ACGIH

Threshold Limit Value - Time-Weighted Average Limit (TLV-TWA) = as low as possible

NIOSH

Listed as a potential occupational carcinogen

REFERENCES

ATSDR. 2001. Toxicological Profile for Benzidine. Update (Final Report). NTIS Accession No. PB/2001/109102. Atlanta, GA: Agency for Toxic Substances and Disease Registry. 218 pp.

ChemSources. 2003. Benzidine. Chemical Sources International, Inc. http://www.chemsources.com and search CAS number 92-87-5.

Choudhary, G. 1996. Human health perspectives on environmental exposure to benzidine: a review. Chemosphere 32(2): 267-91.

HSDB. 2003. Hazardous Substances Database. Benzidine. National Library of Medicine. Last updated: 8/29/03. http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB and search CAS number. Last accessed: 2/20/04.

IARC. 1982. Some Industrial Chemicals and Dyestuffs. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol. 29. Lyon, France: International Agency for Research on Cancer. 416 pp.

IARC. 1987. Overall Evaluations of Carcinogenicity. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Supplement 7. Lyon, France: International Agency for Research on Cancer. 440 pp.

NIOSH. 1984. National Occupational Exposure Survey (1981-83). Cincinnati, OH: U. S. Department of Health and Human Services. http://www.cdc.gov/noes/noes3/empl0003.html.

SRI. 2003. Directory of Chemical Producers. http://dcp.sric.sri.com/Public/ (Visitor Search).

TRI01. 2003. Toxics Chemical Release Inventory 2001. Data contained in the Toxics Chemical Release Inventory (TRI). U. S. Environmental Protection Agency Office of Environmental Information. http://www.epa.gov/triexplorer/.

Dyes Metabolized to Benzidine (Benzidine Dye Class)*

Known to be a human carcinogen
First Listed in the *Ninth Report on Carcinogens* (2000)

Carcinogenicity

Dyes that are metabolized to benzidine are *known to be human carcinogens* based on the following evidence: (1) benzidine is *known to be a human carcinogen*, (2) metabolism of benzidine-based dyes results in the release of free benzidine in humans and in all experimental animal species studied (Rinde and Troll 1975, Lowry *et al.* 1980, Lynn *et al.* 1980, Nony *et al.* 1980, Martin and Kennelly 1985), and (3) benzidine exposure from exposure to benzidine-based dyes is equivalent to exposure to equimolar doses of benzidine (Lynn *et al.* 1980).

The evidence that dyes metabolized to benzidine are human carcinogens is supported by studies showing that all benzidine-based dyes tested cause cancer in experimental animals (NCI 1978, IARC 1982). C.I. direct black 38 administered in drinking water or in the diet caused malignant liver and mammary-gland tumors in mice and malignant liver, colon, and bladder tumors in rats. C.I. direct blue 6 administered in the diet for 13 weeks caused malignant liver tumors in rats but not in mice. In a similar study, C.I. direct brown 95 caused neoplastic nodules in the liver and one malignant liver tumor in rats after 13 weeks. Based on these data, the International Agency for Research on Cancer (IARC) (1987) concluded that there was sufficient evidence for the carcinogenicity of technical-grade C.I. direct black 38, technical-grade C.I. direct blue 6, and technical-grade C.I. direct brown 95 in experimental animals.

Additional Information Relevant to Carcinogenicity

Benzidine was one of the first chemicals for which an association between occupational exposure and increased cancer risk was recognized. Industrial exposure to benzidine was first associated with bladder cancer in the early 1920s. Benzidine was listed as *known to be a human carcinogen* in the *First Annual Report on Carcinogens* (1980). The evidence supporting its listing is summarized in the profile for benzidine, above.

Benzidine was first synthesized in 1845, and the first benzidine-based dye, Congo red, was prepared in 1884. A wide spectrum of colors can be achieved by varying the dye molecules' chromophores, which are linked to benzidine by an azo linkage (–N=N–). Similar or different chromophores may be linked at each amino (NH_2) group of the benzidine molecule to form various bisazobiphenyl dyes. Regardless of the chromophore(s) involved, the azo linkages of all benzidine-based dyes are essentially chemically equivalent; easily formed, they also are easily broken by chemical or enzymatic reduction to form free benzidine and free chromophore(s). Benzidine-based dyes were shown to be metabolized to free benzidine in rats, dogs (Lynn *et al.* 1980), hamsters (Nony *et al.* 1980), and rhesus monkeys (Rinde and Troll 1975), probably by bacteria in the gastrointestinal tract. Lowry *et al.* (1980) concluded that the amount of benzidine and its metabolites detected in urine of exposed workers could not have been accounted for by the minute amounts of free

benzidine in the dyes to which they were exposed, and therefore that humans also metabolize benzidine-based dyes to free benzidine. Lynn *et al.* (1980) found that in rats and dogs, each benzidine-based dye studied was reduced to yield an amount of free benzidine equal to that observed following an equimolar dose of benzidine.

Because benzidine workers exposed to benzidine-based dyes typically have been co-exposed to benzidine, it has been difficult to clearly establish the carcinogenicity of benzidine-based dyes in epidemiological studies. In studies of Chinese workers who remained in the same jobs for many years, You *et al.* (1990) found an increased incidence of bladder cancer in workers exposed almost exclusively to benzidine-based dyes, and Bi *et al.* (1992) found an increased incidence of bladder cancer in workers co-exposed to benzidine and benzidine-based dyes. However, neither report adequately documented levels of exposure to either benzidine or the dyes. IARC (1982) concluded that the epidemiological data were inadequate to evaluate the carcinogenicity of individual benzidine dyes to humans, but that taken together with the presence of benzidine in the urine of exposed workers, they provided sufficient evidence that occupational exposure to benzidine-based dyes increased the risk of cancer in humans.

Properties

All the benzidine-based dyes have the characteristic diazotized benzidine nucleus (the structure is shown in the profile for benzidine, above) but differ with respect to the chemical groups attached at the diazo linkages. Most of the dyes in this class contain two or three azo groups, but they can contain more. They all occur as colored powders (in a wide range of hues) at room temperature and have negligible vapor pressures. Their water solubility varies but is sufficient for dyeing in aqueous solution. Benzidine-based dyes are relatively stable in air and in solution at ambient temperatures but degrade in aqueous solution at high temperatures, particularly in the presence of iron. Impurities, such as benzidine, 4-aminobiphenyl, and 2,4-diaminoazobenzene, may be present in these dyes as a result of thermal or enzymatic decomposition (NIOSH 1980). There are no rigid chemical specifications for benzidine-based dyes; therefore, their composition varies according to the shade and intensity requirements of the customer (IARC 1982). Various dyes also are mixed together to produce particular colors; therefore, the final products are more accurately described as mixtures of substances than as specific chemical compounds (NIOSH 1980).

Use

Benzidine-based dyes were used primarily to color textiles, leather, and paper products and also in the petroleum, rubber, plastics, wood, soap, fur, and hair-dye industries. Approximately 40% was used to color paper, 25% to color textiles, 15% to color leather, and 20% for diverse applications. By the mid 1970s, most manufacturers started phasing out the use of benzidine-based dyes and replacing them with other types of dyes (NIOSH 1980). More than 300 benzidine-based dyes are listed in the Colour Index, including 18 commercially available in the United States. Access to these dyes for home use is no longer permitted in the United States; however, some (particularly direct browns, greens, and blacks) were available as consumer products in the 1970s (ATSDR 2001).

Production

Commercial quantities of benzidine-based dyes were produced in the United States starting no later than 1914, and total U.S. production reached 14 million kilograms (31 million pounds) in 1948 (IARC 1982). In 1974, nine U.S. manufacturers produced benzidine-based dyes, but by 1979, only one manufacturer remained, producing 17 benzidine-based dyes. Domestic production was about 2.9 million kilograms (6.4 million pounds) in 1976 but dropped to about 780,000 kg (1.7 million pounds) in 1978. C.I. direct black 38 accounted for about 48% of U.S. production in 1978, followed by C.I. direct blue 2 (12.8%) and C.I. direct green 6 (6.4%) (NIOSH 1980).

U.S. imports of benzidine-based dyes increased from 272,000 kg (600,000 lb) in 1976 to 730,000 kg (1.6 million pounds) in 1978 (NIOSH 1980) and declined to 213,000 kg (469,000 lb) in 1979. Benzidine-based dyes may still be imported into the United States, but no data on the amounts were found (ATSDR 2001).

Several benzidine-based dyes still have U.S. suppliers, including C.I. direct red 28 (28 suppliers), C.I. direct black 38 (14 suppliers), C.I. direct blue 6 (6 suppliers), C.I. direct green 6 (4 suppliers), C.I. direct brown 95 (2 suppliers), C.I. direct brown 2 (2 suppliers) and C.I. direct blue 2 and C.I. direct black 4 (1 supplier each) (ChemSources 2003). However, these dyes are no longer used or marketed in significant quantities in the United States (ATSDR 2001).

Exposure

The primary routes of potential exposure to benzidine-based dyes are inhalation and accidental ingestion; dermal absorption also can 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 dyes. Since 1980, use of mixtures containing benzidine at concentrations of 0.1% or more is 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). Nevertheless, accidental releases of these dyes could lead to some occupational and environmental exposure (IARC 1982, ATSDR 2001).

In the past, environmental exposure to benzidine-based dyes potentially occurred in the vicinity of dye and pigment plants or waste-disposal sites. According to the U.S. EPA's Toxics Release Inventory (TRI01 2003), no environmental releases of benzidine-based dyes have been reported since 1989, when 750 lb of C.I. direct black 38 was released. The National Occupational Hazard Survey (1972–1974) estimated that 79,200 workers in 63 occupations (primarily the dye manufacturing, textile dyeing, printing, paper, and leather industries) potentially were exposed to benzidine-based dyes (NIOSH 1980). A decade later, the National Occupational Exposure Survey (1981–1983) estimated that about 33,900 workers potentially were exposed to 13 benzidine-based dyes (NIOSH 1984), for a decrease of almost 60%. Although no current estimate of occupational exposure to benzidine-based dyes was found, the number of potentially exposed workers is expected to be much lower than in the past.

Regulations

EPA

Emergency Planning and Community Right-To-Know Act

Toxics Release Inventory: C.I. Direct Blue 6; C.I. Direct Black 38; and C.I. Direct Brown 95 are listed substances subject to reporting requirements

OSHA

C.I. Direct Blue 6; C.I. Direct Black 38; and C.I. Direct Brown 95 should be controlled as carcinogens in the workplace

Guidelines

NIOSH

C.I. Direct Blue 6; C.I. Direct Black 38; and C.I. Direct Brown 95 should be handled in the workplace as if they were human carcinogens

*No separate CAS registry number is assigned to dyes metabolized to benzidine.

REFERENCES

ATSDR. 2001. Toxicological Profile for Benzidine. Update (Final Report). NTIS Accession No. PB/2001/109102. Atlanta, GA: Agency for Toxic Substances and Disease Registry. 218 pp.

Bi, W., R. B. Hayes, P. Feng, Y. Qi, X. You, J. Zhen, *et al.* 1992. Mortality and incidence of bladder cancer in benzidine-exposed workers in China. Am J Ind Med 21(4): 481-9.

ChemSources. 2003. Chemical Sources International, Inc. http://www.chemsources.com.

IARC. 1982. Some Industrial Chemicals and Dyestuffs. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol. 29. Lyon, France: International Agency for Research on

Cancer. 416 pp.
Lowry, L. K., W. P. Tolos, M. F. Boeniger, C. R. Nony and M. C. Bowman. 1980. Chemical monitoring of urine from workers potentially exposed to benzidine-derived azo dyes. Toxicol Lett 7(1): 29-36.
Lynn, R. K., D. W. Donielson, A. M. Ilias, J. M. Kennish, K. Wong and H. B. Matthews. 1980. Metabolism of bisazobiphenyl dyes derived from benzidine, 3,3´-dimethylbenzidine or 3,3´-dimethoxybenzidine to carcinogenic aromatic amines in the dog and rat. Toxicol Appl Pharmacol 56(2): 248-58.
Martin, C. N. and J. C. Kennelly. 1985. Metabolism, mutagenicity, and DNA binding of biphenyl-based azodyes. Drug Metab Rev 16(1-2): 89-117.
NCI. 1978. Thirteen-Week Subchronic Toxicity Studies of Direct Blue 6, Direct Black 38 and Direct Brown 95 Dyes. Techincal Report Series No 108. DHEW (NIH) Publication No. 78-1358. Bethesda, MD: National Institute of Health.
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.
NIOSH. 1984. National Occupational Exposure Survey (1981-83). Cincinnati, OH: U. S. Department of Health and Human Services. http://www.cdc.gov/noes/noes3/empl0003.html.
Nony, C. R., M. C. Bowman, T. Cairns, L. K. Lowry and W. P. Tolos. 1980. Metabolism studies of an azo dye and pigment in the hamster based on analysis of the urine for potentially carcinogenic aromatic amine metabolites. J Anal Toxicol 4(3): 132-40.
Rinde, E. and W. Troll. 1975. Metabolic reduction of benzidine azo dyes to benzidine in the rhesus monkey. J Natl Cancer Inst 55(1): 181-2.
TRI01. 2003. Toxics Chemical Release Inventory 2001. Data contained in the Toxics Chemical Release Inventory (TRI). U. S. Environmental Protection Agency Office of Environmental Information. http://www.epa.gov/triexplorer/.
You, X. Y., J. G. Chen and Y. N. Hu. 1990. Studies on the relation between bladder cancer and benzidine or its derived dyes in Shanghai. Br J Ind Med 47(8): 544-52.