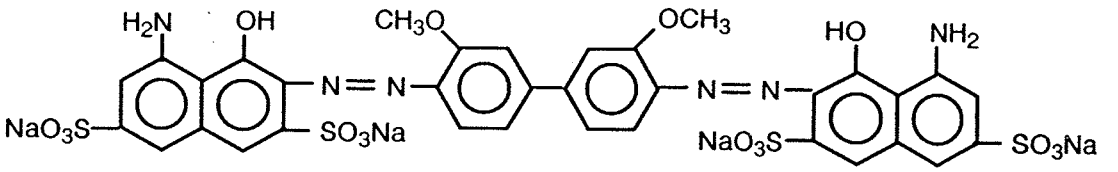
CI DIRECT BLUE 15

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Synonyms, structural and molecular data*

Chem. Abstr. Serv. Reg. No.: 2429-74-5; replaces 51568-94-6; 95032-75-0
Chem. Abstr. Name: 3,3′-[(3,3′-Dimethoxy[1,1′-biphenyl]-4,4′-diyl) bis(azo)]bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid], tetrasodium salt
Colour Index No.: 24400
Synonym: Direct Blue 15



$C_{34}H_{24}N_6O_{16}S_4.4Na$ Mol. wt: 992.85

1.1.2 *Chemical and physical properties*

(*a*) *Description*: Dark-blue powder (US National Toxicology Program, 1992)
(*b*) *Melting-point*: 300 °C (decomposes) (US National Toxicology Program, 1992)
(*c*) *Spectroscopy data*: Infrared and nuclear magnetic resonance spectral data have been reported (US National Toxicology Program, 1992).
(*d*) *Solubility*: Soluble in water; insoluble in most organic solvents (Society of Dyers and Colourists, 1971a)

1.1.3 *Trade names, technical products and impurities*

Some trade names are: Airedale Blue D; Aizen Direct Sky Blue 5B; Aizen Direct Sky Blue 5BH; Amanil Sky Blue; Atlantic Sky Blue A; Atul Direct Sky Blue; Azine Sky Blue 5B; Belamine Sky Blue A; Benzanil Sky Blue; Benzo Sky Blue A-CF; Benzo Sky Blue S; Cartasol Blue 2GF; Chloramine Sky Blue A; Chloramine Sky Blue 4B; Chrome Leather Pure Blue; Cresotine Pure Blue; Diacotton Sky Blue 5B; Diamine Blue 6B; Diamine Sky Blue; Diaphtamine Pure Blue; Diazol Pure Blue 4B; Diphenyl Brilliant Blue; Diphenyl Sky Blue 6B; Direct Blue 10G; Direct Blue HH; Direct Pure Blue; Direct Pure Blue M; Direct Sky

Blue; Direct Sky Blue A; Direct Sky Blue 5B; Enianil Pure Blue AN; Fenamin Sky Blue; Hispamin Sky Blue 3B; Kayafect Blue Y; Kayaku Direct Sky Blue 5B; Mitsui Direct Sky Blue 5B; Naphtamine Blue 10G; Niagara Blue 4B; Niagara Sky Blue; Nippon Direct Sky Blue; Nippon Sky Blue; Nitto Direct Sky Blue 5B; Oxamine Sky Blue 5B; Paper Blue S; Phenamine Sky Blue A; Pontamine Sky Blue 5BX; Shikiso Direct Sky Blue 5B; Sky Blue 4B; Sky Blue 5B; Tertrodirect Blue F; Vondacel Blue HH.

The raw dye contains about 25% sodium chloride; a desalted preparation (containing ~ 3% salt) contained about 50% CI Direct Blue 15 and about 35 impurities, including 3,3′-dimethoxybenzidine dihydrochloride at 836–1310 ppm (mg/kg). Benzidine was not present at the detection limit of 1 ppm (mg/kg) (US National Toxicology Program, 1992).

CI Direct Blue 15 is available at a purity of 65.5%, containing 15 ppm 3,3′-dimethoxybenzidine (*ortho*-dianisidine; see IARC, 1974, 1987) (Bowman *et al.*, 1982).

1.1.4 *Analysis*

No data were available to the Working Group.

1.2 Production and use

1.2.1 *Production*

CI Direct Blue 15 was first prepared in 1890 (Society of Dyers and Colourists, 1971a). It is produced by coupling 3,3′-dimethoxybenzidine to 1-amino-8-naphthol-3,6-disulfonic acid under alkaline conditions (US Environmental Protection Agency, 1987).

Approximate US production was 108 tonnes in 1972, 241 tonnes in 1977, 98 tonnes in 1981 and 123 tonnes in 1982 (US International Trade Commission, 1974, 1978, 1982, 1983).

1.2.2 *Use*

CI Direct Blue 15 is used to dye cellulose, leather, paper, cotton, silk and wool and to stain biological materials; it is also used to tint cinematographic film (Society of Dyers and Colourists, 1971b). The use pattern for CI Direct Blue 15 in the USA is 65% in textile dyeing, 30% as a paper colourant and 5% for other uses.

1.3 Occurrence

1.3.1 *Natural occurrence*

CI Direct Blue 15 is not known to occur as a natural product.

1.3.2 *Occupational exposure*

No data were available to the Working Group.

The US Environmental Protection Agency, the American Textile Manufacturers Institute and the Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry conducted a joint survey in 1986–87 to estimate airborne concentrations of dye dust

in dye weighing rooms of plants where powder dyes are used in the dyeing and printing of textiles. The survey was based on a sample of 24 sites chosen at random from among textile plants where powder dyes are weighed. Although CI Direct Blue 15 was not included in the survey, the results were considered to be representative of dye dust levels during weighing of this type of powder dye. The mean airborne concentration of total active colourant in the plants monitored was estimated to be 0.085 mg/m^3 (US Environmental Protection Agency, 1990).

On the basis of a survey conducted in the USA between 1981 and 1983, the US National Institute for Occupational Safety and Health estimated that a total of 4527 workers, including 201 women, may have been exposed to CI Direct Blue 15 in seven industries (US National Library of Medicine, 1992).

1.3.3 *Other*

Anaerobic biodegradation of CI Direct Blue 15 gives rise to the amine metabolite, 3,3′-dimethoxybenzidine. Following incubation of 100 mg/l of dyestuff at 35 °C in the presence of anaerobic sludge inoculum, primary degradation was complete within seven days (Brown & Hamburger, 1987).

1.4 Regulations and guidelines

In Germany, derived azo dyes must be handled like the corresponding hypothetical reduction products. CI Direct Blue 15 must therefore be handled like 3,3′-dimethoxybenzidine, which is classified as an A2 compound. Those materials are considered to have been proven to be carcinogenic only in animal experimentation but under conditions comparable to those of possible human exposure at the workplace (Deutsche Forschungsgemeinschaft, 1992).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

Rat

Groups of 50, 35, 65 and 50 male and 50, 35, 65 and 50 female Fischer 344/N rats, 40–47 days old, were administered 0, 630, 1250 or 2500 mg/l (ppm) CI Direct Blue 15 (purity, ~ 50%; with ~ 35 impurities, including 3,3′-dimethoxybenzidine) in distilled drinking-water for 96 weeks and were necropsied at 103–104 weeks of age. Survival at 22 months was 37/50, 8/35, 11/65 and 2/50 for male rats and 40/50, 13/35, 22/65 and 4/50 for females in the

control, low-, mid- and high-dose groups, respectively ($p < 0.001$ for both males and females). The decreased survival in the treated groups was due to development of treatment-related neoplasms. As shown in Table 1, there were increased incidences of benign and malignant tumours of the skin, Zymbal gland, liver, oral cavity and small intestines and of mononuclear-cell leukaemia in male and female rats, of benign and malignant tumours of the large intestine and preputial gland in males and of the clitoral gland and uterus in females (US National Toxicology Program, 1992).

Table 1. Survival and tumour incidences in male and female Fischer 344/N rats administered CI Direct Blue 15 in the drinking-water for 96 weeks

Survival and tumour types[a]	Dose (mg/l [ppm])				*p* Value[b]
	0	630	1250	2500	
Males					
Survival[c]	37/50	8/35	11/65	2/50	
Skin					
Basal-cell adenoma or carcinoma	2/50	9/35	27/65	28/50	< 0.001
Sebaceous gland adenoma	0/50	1/35	7/65	3/50	= 0.002
Squamous-cell papilloma or carcinoma	2/50	4/35	11/65	19/50	< 0.001
Zymbal gland: adenoma or carcinoma	1/50	5/35	10/65	20/50	< 0.001
Preputial gland: adenoma or carcinoma	8/49	5/35	23/64	9/48	< 0.001[d]
Hepatocellular neoplasms[f]	0/50	6/35	9/65	11/50	< 0.001
Oral cavity: squamous-cell papilloma or carcinoma	1/50	10/35	24/65	17/50	< 0.001
Small intestine: adenocarcinoma	0/50	0/35	0/65	2/50	= 0.078
Large intestine: polyps or adenocarcinoma	0/50	1/35	6/65	8/50	< 0.001
Mononuclear-cell leukaemia	17/50	19/35	28/65	20/50	< 0.001[d]
Females					
Survival	40/50	13/35	22/65	4/50	
Squamous-cell papilloma or carcinoma of the skin	0/50	2/35	6/65	5/50	= 0.001
Zymbal gland: adenoma or carcinoma	0/50	4/35	11/65	17/50	< 0.001
Clitoral gland: adenoma or carcinoma	7/50	11/31	24/64	27/50	< 0.001
Hepatocellular neoplastic nodule or carcinoma	0/50	0/35	2/65	5/50	< 0.001
Oral cavity: squamous-cell papilloma or carcinoma	2/50	4/35	19/65	15/50	< 0.001
Small intestine: adenocarcinoma	0/50	0/35	1/65	3/50	= 0.032
Uterine adenoma or adenocarcinoma	1/50	0/35	1/65	4/50	= 0.004
Mononuclear-cell leukaemia	7/50	13/35	27/65	15/50	< 0.001[d]

From US National Toxicology Program (1992)

[a]Terms used by authors

[b]Logistic regression trend test

[c]At 22 months; reduced survival in exposed groups due to neoplasia

[d]Life-table test

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Anaerobic biodegradation of CI Direct Blue 15 gives rise to the amine metabolite, 3,3′-dimethoxybenzidine (Brown & Hamburger, 1987). The dye was cleaved by pure cultures of anaerobic bacteria and by suspensions derived from the intestinal content of rats, with subsequent formation of the amine (Cerniglia *et al.*, 1982).

CI Direct Blue 15 (100 mg/kg) containing 46 ppm (mg/kg) 3,3′-dimethoxybenzidine as an impurity was administered once in the diet to two female mongrel dogs weighing 15 kg, and 48-h urine was analysed for 3,3′-dimethoxybenzidine, the potential metabolic product (Lynn *et al.*, 1980). Excretion was found to be 0.03% of the dose of dye administered, which cannot be attributed to the level of impurity. The same dose was also administered once to four male Sprague-Dawley rats by intragastric intubation; after 72 h, 0.17 ± 0.18% of the theoretical maximum was excreted as 3,3′-dimethoxybenzidine and the monoacetyl derivative, the latter constituting a substantial fraction.

When [^{14}C-biphenyl]CI Direct Blue 15 was given as a single dose of 12 mg/kg to six-week-old male Fischer 344 rats by gavage, 74.4% of the dose was excreted in the faeces and 18.8% in urine within 192 h. Only 12% of the dose appeared as intact dye in the faeces within 48 h, the remainder being unidentified metabolic products. Excretion of the free diamine, 3,3′-dimethoxybenzidine, and of its mono- and diacetyl derivatives in urine was determined to be 0.22, 0.27 and 0.22% of the dose, respectively. An equivalent dose of ^{14}C-labelled 3,3′-dimethoxybenzidine was administered for comparison: 52% of the dose appeared in the faeces and 35% in the urine, indicating that the free amine is metabolized to a greater extent than the dye. Only 1.5% of the dose in faeces could be attributed to the free amine fraction, including the acetylated metabolites; in urine, 1.18% of the dose was excreted as the parent compound, 0.35% as the monoacetyl derivative and 0.93% as the diacetyl derivative. Radiolabel was found in all tissues examined from rats dosed with ^{14}C-CI Direct Blue 15. The levels peaked at 4 and 8 h and were highest in the gastrointestinal tract, liver, kidney and lung (Bowman *et al.*, 1982).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental animals*

CI Direct Blue 15 binds to albumin, α_1-lipoprotein, β-lipoprotein, haemopexin, pre-albumin and α_1-antichymotrypsin, to alter their mobility in crossed immunoelectrophoresis and to degrade C_3 globulin (Emmet *et al.*, 1985). The importance of these findings *in vivo* remains to be established, as it is not known how much unchanged dye is absorbed and transported within the body.

CI Direct Blue 15 was tested for subchronic toxicity in male and female Fischer 344 rats (Morgan *et al.*, 1989; US National Toxicology Program, 1992). Groups of 10 animals of each sex received the dye in drinking-water for 13 weeks at concentrations of 0, 0.063, 0.125, 0.25, 0.50 and 1.0% for females and 0, 0.125, 0.25, 0.50, 1.0 and 3.0% for males. Seven male rats died in the highest-dose group; the first death occurred after three weeks and the last after 13 weeks. Groups given 1% CI Direct Blue 15 gained 17% less body weight than controls, and males treated with 3% of the dye gained 43% less weight than controls. Absolute and relative kidney weights increased in a dose-related manner in males and females. Changes in haematology and clinical chemistry were not observed. Histopathology showed renal and hepatic toxicity in high-dose males that died before termination of the study. In addition to necrosis of hepatocytes and fatty metamorphosis, blue pigment was observed in Kupffer cells. Focal necrosis occurred in proximal tubular epithelial cells. Mild chronic nephropathy was observed in male and female rats given 1% of dye.

4.3 Reproductive and prenatal effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see also Table 2 and Appendices 1 and 2)

Technical-grade CI Direct Blue 15 was not mutagenic to *Salmonella typhimurium* in standard protocols, but it was mutagenic under conditions favouring azo reduction, which would generate 3,3′-dimethoxybenzidine, a known mutagen. CI Direct Blue 15 was reported in an abstract to be mutagenic at the *tk* locus in mouse lymphoma L5178Y cells. It did not induce unscheduled DNA synthesis in rat hepatocytes (abstract) or sister chromatid exchange or chromosomal aberrations in Chinese hamster ovary cells *in vitro*.

Activated *ras* genes were found in 21/34 tumours induced in rats by CI Direct Blue 15 (US National Toxicology Program, 1992) and in 1/38 spontaneous tumours tested (Reynolds *et al.*, 1990; Table 3).

Table 2. Genetic and related effects of CI Direct Blue 15

Test system	Result		Dose[a] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SAF, *Salmonella typhimurium*, forward mutation (arabinose resistance)	0	+[b,c]	100.0000	Krishna *et al.* (1986)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	–[d]	250.0000	Elliott & Gregory (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+[e]	62.5000	Elliott & Gregory (1980)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	+[f]	150.0000	Brown & Dietrich (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	0	(+)[g]	150.0000	Brown & Dietrich (1983)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	–	5000.0000	Mortelmans *et al.* (1986)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	–	5000.0000	Mortelmans *et al.* (1986)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	–	5000.0000	Mortelmans *et al.* (1986)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	0	+[d]	125.0000	Reid *et al.* (1984)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+[f]	0.0000	Sugimura *et al.* (1977)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	(+)[d]	500.0000	Elliott & Gregory (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+[e]	62.5000	Elliot & Gregory (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+[f]	150.0000	Brown & Dietrich (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	(+)[g]	150.0000	Brown & Dietrich (1983)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+[h]	50.0000	Prival *et al.* (1984)
SA9, *Salmonella typhimurium* TA98, reverse mutation	0	+[b]	100.0000	Krishna *et al.* (1986)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	–	5000.0000	Mortelmans *et al.* (1986)
URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	0	0.0000	Mirsalis *et al.* (1983); abstr.
G5T, Gene mutation, mouse lymphoma L5178Y cells *in vitro*	–	+	0.0000	Rudd *et al.* (1983); abstr.
SIC, Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	–	2500.0000	Galloway *et al.* 1987)
CIC, Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	–	2500.0000	Galloway *et al.* 1987)

+, positive; (+), weakly positive; –, negative; 0, not tested

[a] μg/ml; 0.0000, not given

[b] Preincubation with hamster or rat liver S9 and flavin mononucleotide supplementation

[c] Rat liver S9 more effective

[d] Anaerobic preincubation or riboflavin supplementation

[e] Plate incorporation and reduction using sodium dithionite

[f] Aerobic preincubation with riboflavin

[g] Anaerobic preincubation with rat caecal bacterial extract, flavin mononucleotide and liver S9

[h] Preincubation with no shaking and hamster liver S9 with flavin mononucleotide

Table 3. Activating *ras* mutations in tumours induced in Fischer 344 rats by CI Direct Blue 15 and in untreated animals

Tumour type	Frequency	N-*ras*	H-*ras*						
			Total	Codon 12		Codon 13		Codon 61	
				GAA	AGA	CGC	GTC	AAA	CTA	CGA
Treated										
Preputial gland adenoma	1/1		1					1		
Preputial gland carcinoma	1/3		1			1				
Clitoral gland carcinoma	8/10	1	7	1		4		2		
Basal-cell carcinoma	5/6	1	4			1	1	1		1
Squamous-cell carcinoma (skin)	6/7		6			2		4		
Mammary fibroadenoma	0/2									
Mammary adenocarcinoma	0/3									
Duodenal adenocarcinoma	0/1									
Subcutaneous fibroma	0/1									
Untreated										
Clitoral gland adenoma	1/2		1							1
Preputial gland carcinoma	0/1									
Mammary gland fibro-adenoma or adenoma	0/11									
Mammary adenocarcinoma	0/2									
Subcutaneous fibroma or fibroadenoma	0/5									
Lipoma	0/1									
Testicular interstitial-cell adenoma	0/5									
Fibrosarcoma	0/2									
Mononuclear-cell leukaemia	0/3									
Adrenal phaeochromocytoma	0/1									
Pancreatic acinar adenoma	0/1									
Pancreatic islet-cell adenoma	0/1									
Pituitary adenoma	0/1									
Splenic haemangiosarcoma	0/1									
Prostatic adenocarcinoma	0/1									

Adapted from Reynolds *et al.* (1990)

5. Summary of Data Reported and Evaluation

5.1 Exposure data

CI Direct Blue 15, a bis-azo dye derived from 3,3′-dimethoxybenzidine, is used mainly for dyeing textiles and paper. The technical grade contains about 50% of pure dye, in addition to inorganic salts and a mixture of about 35 organic compounds, including 3,3′-dimethoxybenzidine.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Technical-grade CI Direct Blue 15 was tested for carcinogenicity in one study in rats by administration in the drinking-water. It produced benign and malignant tumours of the skin, Zymbal gland, liver, small intestine and oral cavity as well as leukaemia in animals of each sex, of the large intestine and preputial gland in males and of the uterus and clitoral gland in females.

5.4 Other relevant data

CI Direct Blue 15 caused renal and hepatic toxicity in rats. Reductive cleavage of the azo bonds to yield 3,3′-dimethoxybenzidine was demonstrated *in vivo*.

CI Direct Blue 15 induced mutation in bacteria under conditions that favour reduction. Neither sister chromatid exchange nor chromosomal aberrations were induced in cultured mammalian cells.

5.5 Evaluation[1]

There is *inadequate evidence* in humans for the carcinogenicity of CI Direct Blue 15.

There is *sufficient evidence* in experimental animals for the carcinogenicity of technical-grade CI Direct Blue 15.

Overall evaluation

CI Direct Blue 15 is *possibly carcinogenic to humans (Group 2B)*.

6. References

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[1]For definition of the italicized terms, see Preamble, pp. 26–30.

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