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GENTIAN VIOLET, LEUCOGENTIAN VIOLET, MALACHITE GREEN, LEUCOMALACHITE GREEN, AND CI DIRECT BLUE 218

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International Agency for Research on Cancer

World Health Organization

CI DIRECT BLUE 218

1. Exposure Characterization

1.1 Identification of the agent

Colour Index (CI) Direct Blue 218 is a bis copper-chelated dimethoxybenzidine-based azo dye. Azo dyes are diazotized amines coupled to an amine or phenol, with one or more azo bonds (R–N=N–R′). The azo group constitutes the chromophore of the dye, i.e. the chemical group primarily responsible for colour (Aspland, 1991). Azo dyes are the most structurally diverse class of organic dyes, with over 3000 azo dyes having been available in the past and 2000 dyes currently available (Chung, 2016).

The essential precursors of azo dyes are aromatic amines (Chung, 2016). CI Direct Blue 218 is based on benzidine or its congeners as precursors (Morgan et al., 1994). Dyes are metallized, in this case with copper, to improve the stability of the azo groups and increase light and wash fastness (Aspland, 1991; Morgan et al., 1994).

CI Direct Blue 218 is a direct dye, also called a substantive dye. Direct dyes have a natural affinity for cellulose without the need for a mordant (Waring & Hallas, 1990; Aspland, 1991). Direct dyes, which mostly belong to the azo class, are superior to others in terms of cost, light fastness, ease of application, short durations of dye cycles, low cost of auxiliaries, remarkably lower use of water, and much lower levels of effluent salt (Textile Property, 2021).

The CI is used to number dyes with respect to their application class and shade using a sequential numbering system (Morgan et al., 1994). The CI constitution number is 24 401 for the dye with the CI generic name CI Direct Blue 218.

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 28407-37-6

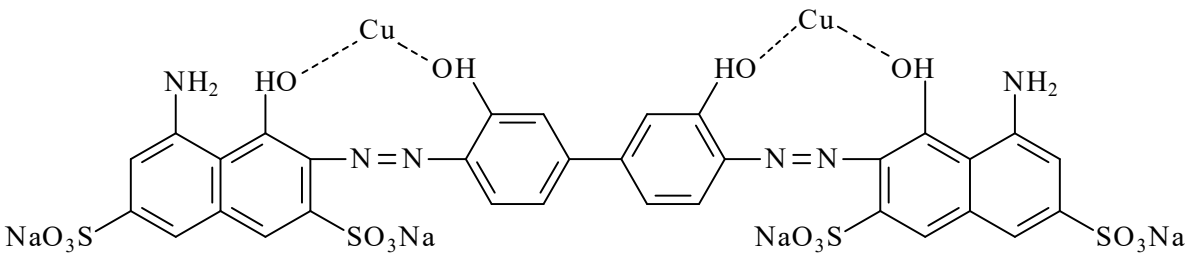
Chem. Abstr. Serv. name: cuprate(4-), [μ-[[3,3′-[[3,3′-di(hydroxy-κO)[1,1′-biphenyl]-4,4′-diyl]bis(2,1-diazenediyl-κN1)]bis[5-amino-4-(hydroxy-κO)-2,7-naphthalenedisulfonato]](8-)]]di-, sodium (1:4)

EC No.: 249-008-8 (ECHA, 2022)

IUPAC systematic name: tetrasodium; 5-amino-3-[[4-[4-[(8-amino-1-hydroxy-3,6-disulfonatonaphthalen-2-yl)diazenyl]-3-hydroxyphenyl]-2-hydroxyphenyl]diazenyl]-4-hydroxynaphthalene-2,7-disulfonate; copper (NCBI, 2020)

Synonyms: DIRECT BLUE 218, 28407-37-6; CI 24 401; Fastusol Blue 9GLP; Solantine Blue 10GL; Pontamine Bond Blue B; Amanil Supra Blue 9GL; CI Direct Blue 218; Pontamine Fast Blue 7GLN; UNII-RR3V5FL20N; RR3V5FL20N; CCRIS 6142; HSDB 4223; NCI C60 877; EINECS 249-008-8;

Fig. 1.1 Chemical structure of CI Direct Blue 218



INTRALITE Blue 8GLL; DIRECTBLUE218; (3,3′-((3,3′-Dihydroxy-1,1′-biphenyl-4,4′-diyl)bis(azo)bis(5-amino-2,7-naphthalenedisulfonato-(O4,O3)))dicopper, tetrasodium salt; 2,7-Naphthalenedisulfonic acid, 3,3′-((3,3′-dihydroxy(1,1′-biphenyl)-4,4′-diyl)bis(azo)bis(5-amino-4-hydroxy-, sodium salt, copper complex; Copper, [mu-[tetrahydrogen-3,3′-[(3,3′-dihydroxy-4,4′-biphenylylene)bis(azo)]bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonato](4-)]di-, tetrasodium salt; Cuprate(4-), [mu-[[3,3′-[(3,3′-dihydroxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonato]](8-)]]di-, tetrasodium; 2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dihydroxy[1,1′-biphenyl]-4,4′-diyl) bis(azo)]bis[5-amino-4-hydroxy-, sodium salt, copper complex; 1-Naphthol-3,6-disulfonic acid, 2,2′-(3,3-dihydroxy-4,4′-dipiphenylylenebisazo)bis[8-amino-, dicopper deriv., tetrasodium salt (CAMEO, 2020; NCBI, 2020).

1.1.2 Structural and molecular formulae, and relative molecular mass

The chemical structure of CI Direct Blue 218 is provided in Fig 1.1.

Molecular formula: $C_{32}H_{20}Cu_2N_6Na_4O_{16}S_4$ (PubChem CID: 34237 or 137241407)

Relative molecular mass: 1091.9 (powder form)

A solution form of this dye is indicated as PubChem CID: 24832073, with the molecular formula $C_{32}H_{16}Cu_2N_6Na_4O_{16}S_4$ and a relative molecular mass of 1087.8.

[The Working Group noted that the chemical and physical properties presented below are for the powder form.]

1.1.3 Chemical and physical properties of the pure substance

Description: deep purple to dark blue amorphous powder (NTP, 1992; ECHA, 2020a)

Boiling point: 1560 °C (estimated, based on EPI Suite MPVPBP V1.43) (ECHA, 2020a)

Melting point: 350 °C (estimated, based on EPI Suite MPVPBP V1.43) (ECHA, 2020a)

Density: 2.8 ± 0.1 g/cm^3 at 20 °C (ECHA, 2020a)

Solubility: 1.00–5.00 × 10^{-4} mg/L at 17 °C in water (ECHA, 2020a)

Vapour pressure: 1.06 × 10^{-39} Pa at 25 °C (ECHA, 2020a)

Flash point: flash-point data for this chemical are not available; however, it is probably combustible (NTP, 1992)

Stability and reactivity: CI Direct Blue 218 is a diazo compound. Azo, diazo, and azido compounds can detonate. Their nitro groups facilitate rapid decomposition. This particularly applies to organic azides that have been sensitized by the addition of metal salts or strong acids. Toxic gases are formed by mixing materials of this class with acids, aldehydes, amides, carbamates, cyanides, inorganic fluorides, halogenated organics, isocyanates, ketones, metals, nitrides, peroxides, phenols, epoxides, acyl halides, and strong oxidizing or reducing agents. Flammable gases are formed by mixing materials in this group with alkali metals. Explosive combination can occur with strong oxidizing agents, metal salts, peroxides, and sulfides (CAMEO, 2020).

Octanol/water partition coefficient (P): log K_{ow}, −0.77 (ECHA, 2020a).

1.1.4 Impurities

The purity of CI Direct Blue 218 was reported to be approximately 60% in a study by the National Toxicology Program (NTP) (NTP, 1994). Chemical characterization of two lots characteristic of the product used by industry indicated more than 12 impurities, of which the majority appeared to contain the reducible azo bond (Morgan et al., 1994; NTP, 1994). No attempt was made to identify the chromatographic peaks; however, reduction titration of azo groups indicated that the two lots had purities of 90% and 83%. The concentrations of benzidine and 3,3′-dimethoxybenzidine were determined. Benzidine could not be detected in either lot at levels greater than 1 ppm [1 µg/mL]. 3,3′-Dimethoxybenzidine was found at levels less than or equal to 7 ppm [7 µg/mL], but the level was less than 1 ppm in the lot used for a 2-year bioassay (Morgan et al., 1994; NTP, 1994). The study by the NTP also noted that other impurities, in addition to those containing azo groups, probably included inorganic copper salts.

[The Working Group noted that the only data on impurities were derived from the above-mentioned assays reported by the NTP in 1994. However, several manufacturers listing CI Direct Blue products online in 2021 were claiming a purity of 96–99%.]

1.2 Production and use

1.2.1 Production process

CI Direct Blue 218 is produced by coupling one mole of *ortho*-dianisidine (3,3′-dimethoxybenzidine) to two moles of 4-amino-5-hydroxy-2,7-naphthalene disulfonic acid under alkaline pH conditions (resulting in CI Direct Blue 15), followed by the addition of a copper salt and the elimination of methyl groups from the methoxides to form a copper complex (Kirk-Othmer, 1978). Due to the copper chelation process, the substance does not contain methoxy groups that are characteristic of the *ortho*-dianisidine (3,3′-dimethoxybenzidine) moiety, which is a major component of the dye (NIOSH, 1980).

1.2.2 Production volume

CI Direct Blue 218 is listed by the Organisation for Economic Co-operation and Development (for the year 2007) as a High Production Volume chemical (OECD, 2009). The production volumes in the USA were 4.03×10^5 kg in 1977, 3.54×10^5 kg in 1979, and 3.33×10^5 kg in 1985 (NCBI, 2003). In 1994, there were four production plants registered in the USA (NCBI, 2003).

Production or import volumes in the USA were reported to be between 10 000 and 500 000 pounds [between 4.5 and 230 tonnes] in 1986, 1990, 1994, 1998, and 2002, and < 500 000 pounds [< 230 tonnes] in 2006 (US EPA, 2003, 2007; IARC, 2010). The national aggregated production volumes in the USA

according to Chemical Data Reporting records were < 1 000 000 pounds [< 450 tonnes] per year from 2012 to 2015 (US EPA, 2020). In 2020, CI Direct Blue 218 was available from two suppliers in the USA and 12 suppliers from China (Chemical Register, 2020). Based on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) registration data reported in 2018, between 1 and 10 tonnes of CI Direct Blue 218 were manufactured or imported in the European Economic Area (ECHA, 2020a).

[The Working Group noted that no specific information could be found on production volumes of CI Direct Blue 218 in China or elsewhere outside of the USA.]

1.2.3 Use

CI Direct Blue 218 is used as a dye for cellulose, acetate, nylon, silk, wool, tissue, fine papers, and textile goods with a urea-formaldehyde finish (NCBI, 2003; ECHA, 2020a). As noted in the introduction to this section, direct dyes have a natural affinity for cellulose without the need for a mordant (Waring & Hallas, 1990; Aspland, 1991). Azo dyes are synthetic compounds with vivid colours, of which > 7 × 10^5 tons [> 6.4 × 10^5 tonnes] are produced annually worldwide, accounting for > 50% of all dyestuffs produced worldwide (Chung, 2016). Boeniger (1980) reported the total use of benzidine-based dyes as follows: 40% to colour paper, 25% to colour textiles, 15% to colour leather, and 20% in diverse applications in the petroleum, rubber, plastics, wood, soap, fur, and hair dye industries (Boeniger, 1980). [The Working Group noted that these data were not specific to CI Direct Blue 218, for which no data were available.]

1.3 Methods of detection and quantification

1.3.1 Air

No specific methods are available for the measurement of CI Direct Blue 218 in air. National Institute for Occupational Safety and Health (NIOSH) method No. 5013 is available for benzidine-based dyes (NIOSH, 1994). This method also can be used for *ortho*-dianisidine-based (3,3′-dimethoxybenzidine-based) dyes. Sampling of airborne dyes is performed with a 5 µm polytetrafluoroethylene membrane filter with a flow rate of 1–3 L/minute. After ultrasonic treatment of the filter, sodium hydrosulfite is added for reductive cleavage of the dye; the isolated benzidine is finally analysed by high-performance liquid chromatography-ultraviolet (HPLC-UV). The estimated limit of detection (LOD) of the method is 6–20 µg/m^3.

1.3.2 Other environmental media

Few specific methods are available for the measurement of CI Direct Blue 218 and dimethoxybenzidine-based dyes in environmental media. A method for the chemical characterization of CI Direct Blue 218 was used that applied thin-layer chromatography and HPLC with UV/visible light detection at 254 and 658 nm (NTP, 1994). CI Direct Blue 218 was also measured in feed following extraction with methanol or methanol with tetrabutylammonium hydroxide by spectrophotometric measurement of the 622 nm absorbance maximum. Different analytical methods are used for the analysis of 3,3′-dimethoxybenzidine and dimethoxybenzidine-based dyes in a variety of matrices (water, paint, textiles, food, and toys). In general, methods involve reductive cleavage of the dye to the free amine and analysis of the resultant amines. [The Working Group noted that, in the case of CI Direct Blue, it is unlikely that 3,3′-dimethoxybenzidine is

formed after cleavage, since the methoxy groups are no longer present. Instead 3,3′-dihydroxybenzidine is more likely to be formed. The analytical methods described below are also relevant for these products because they detect the amine groups.]

While gas chromatography (GC) analysis invariably requires derivatization of the amine before the analysis, analysis by liquid chromatography (LC) in combination with mass spectrometry (MS) does not. Also, the use of modern liquid chromatography with tandem mass spectrometry (LC-MS/MS) methods permits the analysis of complex mixtures. A method based on the analysis of amine with HPLC-UV (after reductive cleavage) has been developed for 3,3′-dimethoxybenzidine-based dyes in toys, with an estimated LOD of 0.2 µg/g (Garrigós et al., 2002). For wastewater, a method has been developed by dissolving the dyes in methanol, dichloromethane, or ethylacetate before analysis with GC-MS (Doherty, 2005). For textiles, a method that involves refluxing with chlorobenzene followed by three consecutive extractions with citrate buffer, hydrosulfite, and *tert*-methyl ether was developed for use with LC-MS/MS analysis (Sutthivaiyakit et al., 2005). This method forms the basis for the later-developed International Organization for Standardization (ISO) method 14362-1:2017 (ISO, 2017). In the ISO method, several chromatographic techniques and detection methods are described: HPLC with diode-array detection, HPLC-MS, GC-MS, and GC-flame ionization detection. The estimated LOD of the method is 5 µg/g.

1.3.3 Biological specimens

No biomonitoring methods for the assessment of exposure to CI Direct Blue 218 or its possible metabolites were identified in the literature.

1.4 Occurrence and exposure

1.4.1 Environmental occurrence

A study of the Yamaska River in Quebec, Canada, conducted between 1985 and 1987, investigating the occurrence of 15 dyes in river water, sediments, and solids, as well as fish downstream of textile mills, did not detect CI Direct Blue 218 (Maguire, 1992). No other environmental studies on CI Direct Blue 218 were identified. Very little is known about the environmental occurrence, persistence, and fate of individual dyes because of difficulties in determining different chemical classes of dyes at trace levels in environmental samples (Maguire, 1992). Benzidine and its congeners are not known to occur naturally in the environment. Although few actual measurements of the release of benzidine-based compounds into the environment have been reported, it is thought that manufacturing and processing plants for dyes and pigments derived from benzidine and its congeners are the major sources of release. Three major sources of environmental release of dyes and pigments derived from benzidine and its congeners have been identified: process wastewaters; atmospheric release; and disposal of dyed articles (US EPA, 1980).

About 10% of the azo dyes used in textile dyeing processes are released into the environment (Chung, 2016; dos Santos, 2018). In the 1980s, it was estimated that, worldwide, 280 000 tons [units assumed to be imperial tons; 284 494 metric tonnes] of textile dyes were annually discharged into industrial effluents (Chung, 2016). Since the azo dyes represent about 70% by weight of the dyestuffs used, it follows that they are the most common group of synthetic colourants released into the environment (Chung, 2016).

Generally, ionic azo dyes released into surface waters or wastewater are expected to bind primarily to suspended organic matter,

due to electrostatic interactions, and ultimately sequester to sediments or wastewater sludge. However, a proportion of ionic dyes are likely to remain dissolved in the water column (dos Santos, 2018). Because of their stability and microbial resistance, azo dyes are not readily removed from wastewater by conventional treatment methods (Chung, 2016). Contaminated sewage sludge is often dumped in landfill waste sites, resulting in soil and groundwater contamination (Tkaczyk et al., 2020). Furthermore, sludge from wastewater treatment plants or industrial wastewater may be applied to agricultural fields, which may result in significant concentrations of dyes in agricultural soils (dos Santos, 2018; Tkaczyk et al., 2020). Complexed metal dyes have half-lives of 2–13 years and in the aquatic environment the heavy metal cations can be assimilated by fish gills, which can lead to accumulation in certain tissues (Lellis et al., 2019). As a consequence of both the direct and indirect sources described above, synthetic organic dyes pass through different trophic levels of the food web, from (water) plants and algae through consumers of the first order (e.g. crustaceans) and through secondary consumers (e.g. fish) to humans. Since this may cause biomagnification of dye components in the food chain, these compounds are considered to be persistent bioaccumulative toxic substances (Tkaczyk et al., 2020).

1.4.2 Occupational exposure

From a survey conducted between 1981 and 1983, NIOSH estimated that a total of 12 290 workers might come into contact with CI Direct Blue 218 in the textile and paper industries (NIOSH, 2017). Industrial exposure to dyes may occur through inhalation of dust or mist, accidental ingestion, or direct contact with the skin (NIOSH, 1980). Potential exposure to CI Direct Blue 218 occurs during the manufacturing process (synthesis, processing, packaging, transportation, or maintenance and clean-up), from the application of the dye on products, and from further processing of dyed products that results in particles being formed (NIOSH, 1983).

1.4.3 Exposure in the general population

No data on exposure to CI Direct Blue 218 in the general population have been identified; however, most environmental exposure to 3,3′-dimethoxybenzidine and 3,3′-dimethoxybenzidine-based dyes has been described as resulting from contact with contaminated air, water, or soil. In addition, the general population may also be exposed to 3,3′-dimethoxybenzidine-based dyes via contact with paper or fabric products containing these dyes, or through consumer use of the dyes (NTP, 2016). However, the benzidine-based dyes in finished products are not considered to migrate from the product as a result of washing, perspiration, or contact with saliva (US EPA, 1980).

1.5 Regulations and guidelines

CI Direct Blue 218 is very toxic to aquatic life with long-lasting effects (H410) and causes serious eye irritation (H319) (ECHA, 2020a). There are additional regulations and guidelines for overarching groups of dyes to which CI Direct Blue 218 may belong. For dimethoxybenzidine-based azo dyes, these are Cosmetics Directive 1223/2009/EC (European Commission, 2009) and the Opinion of the Scientific Committee on Cosmetic and Non-Food Products intended for Consumers (SCCNFP) (SCCNFP, 2002). For dyes that are metabolized to aromatic amines, the following are available: REACH Annex XVII (ECHA, 2020b); Plastic Food Contact Materials Directive 10/2011/EC (European Commission, 2011); Toys Safety Directive: EN-71 standards (European Committee for Standardization, 2014); and guidelines from the United States Occupational Safety and Health Administration (OSHA) and NIOSH (NIOSH, 1980).

2. Cancer in Humans

No data were available to the Working Group.

3. Cancer in Experimental Animals

See Table 3.1.

3.1 Mouse

Oral administration (feed)

In a study of chronic toxicity and carcinogenicity that complied with Good Laboratory Practice (GLP) and that was conducted by the NTP (1994), groups of 50–51 male and 50 female B6C3F$_1$ mice (age, 7 weeks) were given feed containing CI Direct Blue 218 (a desalted commercial dye of 60% copper complex of 3,3′-[(3,3′-dihydroxy[1,1′-biphenyl]-4,4′-diyl) bis(azo)]bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid] tetrasodium salt; 11% water, 0.7% sodium chloride; reduction titration of azo groups indicated a purity of 83%, and the authors stated that the titration estimate of purity was "probably enhanced by the presence of reducible low molecular weight organic impurities containing the azo group as well as inorganic copper salts"; 3,3′-dimethoxybenzidine was < 1 ppm, and benzidine was ≤ 1 ppm) at a concentration of 0, 1000, 3000, or 10 000 ppm (approximately equal to average daily doses of 0, 120, 360, and 1520 mg/kg body weight (bw) per day for males and 0, 140, 470, and 2050 mg/kg bw per day for females, respectively), for the control group and groups at the lowest, intermediate, and highest dose, respectively, for 104 weeks. In addition, 9 males for the control group and 10 males and 10 females for all the other groups were given feed containing CI Direct Blue 218 at a concentration of 0 (controls), 1000, 3000, or 10 000 ppm, respectively, for interim evaluation at 15 months. Survival of exposed male and female mice was similar to that of the controls. At study termination (104 weeks), survival was 44/50, 46/50, 42/50, and 45/50 in males, and 37/49, 40/50, 46/49, and 38/49 in females, for the control group and groups at the lowest, intermediate, and highest dose, respectively. The final mean body weight of mice at the highest dose was 10 000 ppm was 19% lower than that of the control mice for males and 27% lower than that of the control mice for females. Complete necropsies and full histopathological examinations were performed.

In male mice, there was a significant positive trend in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) ($P < 0.001$, logistic regression trend test), with a significant increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) at the highest dose ($P < 0.001$; $P = 0.019$; $P < 0.001$, respectively, logistic regression test). Renal tubule adenomas were observed in two males at the lowest dose, one male at the intermediate dose, and one male at the highest dose. In addition, one renal tubule carcinoma was observed in another male at the lowest dose. No renal tubule tumours were observed in mice in the control group. The incidence of renal tubule adenoma or carcinoma (combined) in historical controls was 4/1366 (0.3 ± 0.7%; range, 0–2%), and only one renal tubule carcinoma was observed. [The Working Group noted that although there was no statistically significant increase, the renal tubule tumours may have been treatment-related because these tumours are rare in this strain of mouse.] Carcinomas of the small intestine occurred in three males at the highest dose and in one mouse in the control group. In addition, in the 15-month interim evaluation experiment, one carcinoma of the small intestine was observed in a male at the highest dose, but none were reported for the controls. In the historical

Table 3.1 Studies of carcinogenicity with CI Direct Blue 218 in experimental animals

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) 7 wk 104 wk NTP (1994)	Oral CI Direct Blue 218, copper complex of 3,3′-[(3,3′-dihydroxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)] bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid] tetrasodium salt, 60%; reduction titration of azo groups indicated purity of 83%, and the authors stated that the titration estimate of purity was probably enhanced by the presence of reducible low molecular weight organic impurities containing the azo group as well as inorganic copper salts; 3,3′-dimethoxybenzidine, < 1 ppm; benzidine, ≤ 1 ppm Feed 0, 1000, 3000, 10 000 ppm 50, 50, 50, 50 44, 46, 42, 45	*Liver* Hepatocellular adenoma 16/50, 19/50, 17/50, 40/50* Hepatocellular carcinoma 7/50, 3/50, 8/50, 17/50* Hepatocellular adenoma or carcinoma (combined) 21/50, 20/50, 23/50, 45/50* *Kidney* Renal tubule adenoma 0/50, 2/50, 1/50, 1/50 Renal tubule carcinoma 0/50, 1/50, 0/50, 0/50 Renal tubule adenoma or carcinoma (combined) 0/50, 3/50, 1/50, 1/50 *Small intestine (jejunum)*: carcinoma 1/50, 0/50, 0/50, 3/50	 $P < 0.001$ (trend), *$P < 0.001$, all logistic regression tests $P < 0.001$ (trend), *$P = 0.019$, all logistic regression tests $P < 0.001$ (trend), *$P < 0.001$, all logistic regression tests NS NS NS NS	Principal strengths: complied with GLP; adequate duration of exposure and observation; used males and females Principal limitations: impurity of the test compound Incidence in historical controls: renal tubule adenoma or carcinoma (combined), 4/1366 (0.3 ± 0.7%; range, 0–2%); renal tubule adenoma, 3/1366 (0.2 ± 0.6%; range, 0–2%); renal tubule carcinoma, 1/1366 (0.1 ± 0.4%; range, 0–2%); small intestine adenoma, adenomatous polyp, or carcinoma (combined), 12/1374 (0.9 ± 1.0%; range, 0–4%); small intestine adenoma or adenomatous polyp, 5/1374 (0.4 ± 1.0%; range, 0–4%); small intestine carcinoma,7/1374 (0.5 ± 1.0%; range, 0–4%) Final mean body weight of group at the highest dose was 19% lower than that of control group

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (F) 7 wk 104 wk NTP (1994)	Oral CI Direct Blue 218, copper complex of 3,3′-[(3,3′-dihydroxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)] bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid] tetrasodium salt, 60%; reduction titration of azo groups indicated purity of 83%, and the authors stated that the titration estimate of purity was probably enhanced by the presence of reducible low relative molecular mass organic impurities containing the azo group as well as inorganic copper salts; 3,3′-dimethoxybenzidine, < 1 ppm; benzidine, ≤ 1 ppm Feed 0, 1000, 3000, 10 000 ppm 49, 50, 49, 49 37, 40, 46, 38	*Liver* Hepatocellular adenoma 7/49, 12/50, 17/49*, 41/49** Hepatocellular carcinoma 5/49, 5/50, 6/49, 12/49 (24%) Hepatocellular adenoma or carcinoma (combined) 10/49, 15/50, 21/49*, 45/49**	$P < 0.001$ (trend), *$P = 0.041$, **$P < 0.001$, all logistic regression tests $P = 0.012$ (trend), logistic regression test $P < 0.001$ (trend), *$P = 0.045$, **$P < 0.001$, all logistic regression tests	Principal strengths: complied with GLP; adequate duration of exposure and observation; used males and females Principal limitations: impurity of the test compound Incidence in historical controls hepatocellular carcinoma, 80/1363 mice (range, 0–20%) Final mean body weight of the group at the highest dose was 27% lower than that of control group

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344/N (M) 6–7 wk 103 wk NTP (1994)	Oral CI Direct Blue 218, copper complex of 3,3′-[(3,3′-dihydroxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)] bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid] tetrasodium salt, 60%; reduction titration of azo groups indicated purity of 83%, and the authors stated that the titration estimate of purity was probably enhanced by the presence of reducible low molecular weight organic impurities containing the azo group as well as inorganic copper salts; 3,3′-dimethoxybenzidine, < 1 ppm; benzidine, ≤ 1 ppm Feed 0, 1000, 3000, 10 000 ppm 50, 50, 50, 51 30, 25, 29, 24	*Oral epithelium (pharynx)* Squamous cell papilloma 0/50, 0/50, 0/50, 5/50* Squamous cell carcinoma 0/50, 0/50, 0/50, 1/50 Squamous cell papilloma or carcinoma (combined) 0/50, 0/50, 0/50, 6/50* *Forestomach* Squamous cell papilloma 0/50, 0/50, 2/50, 1/50 Squamous cell carcinoma 0/50, 0/50, 1/50, 0/50 Squamous cell papilloma or carcinoma (combined) 0/50, 0/50, 3/50, 1/50	 $P < 0.001$ (trend), *$P = 0.026$, all logistic regression tests NS $P < 0.001$ (trend), *$P = 0.013$, all logistic regression tests NS NS NS	Principal strengths: complied with GLP; adequate duration of exposure and observation; used male and females Principal limitations: impurity of the test compound Incidence in historical controls: oral epithelium squamous cell papilloma or carcinoma (combined), 10/1253 (1.4 ± 0.8%; range, 0–4%); oral epithelium squamous cell papilloma, 10/1253 (1.4 ± 0.8%; range, 0–4%); oral epithelium squamous cell carcinoma, 0/1253; forestomach basal cell hyperplasia, significantly increased in treated groups; forestomach squamous cell papilloma or carcinoma (combined), 4/1253 (0.3 ± 0.8%; range, 0–2%); forestomach squamous cell papilloma, 3/1253 (0.2 ± 0.6%; range, 0–2%); forestomach squamous cell carcinoma (combined), 1/1253 (0.1 ± 0.4%; 0–2%) Final mean body weight of group at highest dose was 11% lower than that of control group

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Agent tested, purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence of tumours	Significance	Comments
Full carcinogenicity Rat, F344/N (F) 6–7 wk 103 wk NTP (1994)	Oral CI Direct Blue 218, copper complex of 3,3′-[(3,3′-dihydroxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)] bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid] tetrasodium salt, 60%; reduction titration of azo groups indicated purity of 83%, and the authors stated that the titration estimate of purity was probably enhanced by the presence of reducible low relative molecular mass organic impurities containing the azo group as well as inorganic copper salts; 3,3′-dimethoxybenzidine, < 1 ppm; benzidine, ≤ 1 ppm Feed 0, 1000, 3000, 10 000 ppm 51, 51, 50, 50 35, 29, 31, 25	*Uterus*: endometrial stromal polyp 1/50, 12/50*, 10/50**, 10/50***	*$P < 0.001$, **$P = 0.004$, ***$P = 0.001$, all logistic regression tests	Principal strengths: complied with GLP; adequate duration of exposure and observation; used males and females Principal limitations: impurity of the test compound Incidence in historical controls: endometrial stromal polyps, 205/1251 (16.4 ± 6.6%; range, 2–30%) Final mean body weight of group at the highest dose was 9% lower than that of control group
		Oral epithelium (pharynx): squamous cell papilloma 1/50, 1/50, 0/50, 2/50	NS	
		Forestomach: squamous cell papilloma 0/50, 0/50, 0/50, 1/50	NS	

F, female; GLP, Good Laboratory Practice; M, male; NS, not significant; ppm, parts per million; wk, week.

controls, the incidence of carcinoma of the small intestine was 7/1374 (0.5 ± 1.0%; range, 0–4%).

In female mice, there was a significant positive trend in the incidence of hepatocellular adenoma ($P < 0.001$, logistic regression trend test), hepatocellular carcinoma ($P = 0.012$, logistic regression trend test), and hepatocellular adenoma or carcinoma (combined) ($P < 0.001$, logistic regression trend test), with a significant increase in the incidence of hepatocellular adenoma (intermediate dose, $P = 0.041$; highest dose, $P < 0.001$; logistic regression tests) and hepatocellular adenoma or carcinoma (combined) (intermediate dose, $P = 0.045$; highest dose, $P < 0.001$; logistic regression tests).

Regarding non-neoplastic lesions, there was a significant increase in the incidence of eosinophilic foci of the liver at the highest dose in males, and a significant increase in the incidence of clear cell foci, eosinophilic foci, and foci (all) of the liver at the highest dose in females.

[The Working Group noted this was a well-conducted study that complied with GLP, males and females were used, and the duration of exposure and observation was adequate. The impurity of the compound used was a weakness of the study. The Working Group noted that, of the impurities, benzidine and 3,3′-dimethoxybenzidine were present at low levels.]

3.2 Rat

Oral administration (feed)

In a study of chronic toxicity and carcinogenicity that complied with GLP, four groups of 50–51 male and four groups of 50–51 female F344/N rats (age, 6–7 weeks) were given feed containing CI Direct Blue 218 at concentrations of 0, 1000, 3000, or 10 000 ppm (representing average daily doses of approximately 0, 40, 120, and 440 mg/kg bw per day for males, and 0, 50, 140, and 470 mg/kg bw per day for females, respectively) for the control group and groups at the lowest, intermediate, and highest dose, respectively, for 103 weeks (NTP, 1994). The agent was a desalted commercial dye of 60% copper complex of 3,3′-[(3,3′-dihydroxy[1,1′-biphenyl]-4,4′-diyl) bis(azo)]bis[5-amino-4-hydroxy-2,7-naphthalenedisulfonic acid] tetrasodium salt; 11% water, 0.7% sodium chloride; reduction titration of azo groups indicated a purity of 83%, and the authors stated that the titration estimate of purity was probably "enhanced by the presence of reducible low molecular weight organic impurities containing the azo group as well as inorganic copper salts"; 3,3′-dimethoxybenzidine was < 1 ppm, and benzidine was ≤ 1 ppm. Survival of females at the highest dose was slightly reduced, but not significantly lower than that of the control group. At study termination, survival was 30/50, 25/50, 29/50, and 24/51 for males, and 35/51, 29/51, 31/50, and 25/50 for females for the control group and groups at the lowest, intermediate, and highest dose, respectively. Final mean body weights of rats at the highest dose was 11% lower than that of the controls for males, and 9% lower than that of the controls for females. Complete necropsies and full histopathological examinations were performed.

In male rats, there was a significant positive trend in the incidence of squamous cell papilloma of the oral epithelium (pharynx) ($P < 0.001$, logistic regression trend test), with a significant increase in incidence at the highest dose ($P = 0.026$, logistic regression test). In addition, one squamous cell carcinoma of the oral epithelium (pharynx) was also observed in one rat at the highest dose; no squamous cell carcinomas of the oral epithelium were observed in 1253 male historical controls. There was a significant positive trend in the incidence of squamous cell papilloma or carcinoma (combined) of the oral epithelium (pharynx) ($P < 0.001$, logistic regression trend test), with a significant increase in incidence at the highest dose ($P = 0.013$, logistic regression test). While there was no significant increase in the incidence of forestomach squamous cell

papilloma or carcinoma (combined), the incidence at the intermediate dose – control group, 0/50; lowest dose, 0/50; intermediate dose, 3/50 (6%); and highest dose, 1/50 (2%) – exceeded the upper bound of the range observed in historical controls in this laboratory (4/1253, 0.3 ± 0.8%; range, 0–2%). [Since the incidence of basal cell hyperplasia in the forestomach was significantly increased at the two higher doses in males, and forestomach tumours are rare in this strain of rat, the Working Group considered that the increased incidence of forestomach tumours may have been treatment-related.]

In female rats, the incidence of uterine endometrial stromal polyps – control group, 1/50 (2%); lowest dose, 12/50 (24%); intermediate dose, 10/50 (20%); highest dose, 10/50 (20%) – was significantly increased in each treated group compared with controls (lowest dose, $P < 0.001$; intermediate dose, $P = 0.004$; highest dose, $P = 0.001$, logistic regression test). However, there was no dose–response relationship and, when compared with the incidence and range in historical controls (205/1251, 16.4 ± 6.6%; range, 2–30%), the incidence in the concurrent controls (1/50, 2%) was very low. [The Working Group considered that the higher incidence of endometrial stromal polyps in the exposed groups was probably not treatment-related.]

Regarding non-neoplastic lesions, there was a significant increase in the incidence of basal cell hyperplasia of the forestomach in males at the intermediate and highest dose.

[The Working Group noted this was a well-conducted study that complied with GLP, males and females were used, and the duration of exposure and observation was adequate. The impurity of the compound used was a weakness of the study. The Working Group noted that, of the impurities, benzidine and 3,3′-dimethoxybenzidine were present at low levels.]

3.3 Evidence synthesis for cancer in experimental animals

The carcinogenicity of CI Direct Blue 218 has been assessed in one study in male and female mice and in one study in male and female rats exposed by oral administration (in the feed).

In a study that complied with GLP, male and female B6C3F$_1$ mice were treated with CI Direct Blue 218 in the feed (NTP, 1994). In male mice, there was a significant positive trend and significant increase in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined). In female mice, there was a significant positive trend in the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined), with a significant increase in the incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined).

In a study that complied with GLP, male and female F344/N rats were treated with CI Direct Blue 218 in the feed (NTP, 1994). In male rats, there was a significant positive trend and significant increase in the incidence of squamous cell papilloma of the oral epithelium (pharynx), and squamous cell papilloma or carcinoma (combined) of the oral epithelium (pharynx). In female rats, a statistically significant increase in the incidence of uterine endometrial stromal polyps was probably not treatment-related.

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

No data on the absorption, distribution, metabolism, or excretion of CI Direct Blue 218 in mammalian systems were available to the Working Group. [Based on data from bacterial

mutagenicity assays (see Section 4.2.1(b)(iii)), the Working Group noted that the azo bond of CI Direct Blue 218 could be reduced, generating 3,3′-dihydroxybenzidine or its respective copper-complexed form.]

4.2 Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), including whether CI Direct Blue 218 is genotoxic or causes immortalization. Insufficient data were available for the evaluation of other key characteristics of carcinogens. No data relevant to the key characteristics of carcinogens were available from the Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes in the USA because CI Direct Blue 218 was not tested in this assay battery.

4.2.1 Is genotoxic

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

(i) Non-human mammals in vivo

No data were available to the Working Group.

(ii) Non-human mammals in vitro

See Table 4.1.

CI Direct Blue 218 induced mutations in the presence, but not in the absence, of metabolic activation in a mouse lymphoma assay (Mitchell et al., 1997). Negative results were obtained when the ability of CI Direct Blue 218 to induce chromosomal aberrations was tested in Chinese hamster ovary cells with and without metabolic activation (NTP, 2020). It significantly increased the frequency of sister-chromatid exchanges compared with the negative control without, but not with, metabolic activation. [The Working Group noted that no information on purity was provided in these studies.]

(iii) Non-mammalian experimental systems

See Table 4.2.

Woodruff et al. (1985) reported that CI Direct Blue 218 did not induce sex-linked recessive mutation in meiotic and postmeiotic germ cell stages of Canton-S male *Drosophila melanogaster* treated with CI Direct Blue 218 in the feed or via injection. [The Working Group noted that no information on purity was provided.]

Results were largely negative in the available *Salmonella*/microsome mutagenicity assays. Gregory et al. (1981) reported negative results in tests performed under different reductive conditions (addition of riboflavin, nitrogen gas, and sodium dithionate) in the presence of metabolic activation in strains TA98 and TA100. In tests performed in the presence of sodium dithionate in strain TA100, more than double the numbers of revertants per plate in comparison with the negative control were obtained at the three lowest doses tested, whereas results at the two highest doses were comparable with those for the negative controls. [The Working Group noted that the experiment was not repeated, so no conclusion on the mutagenicity of CI Direct Blue 218 in TA100 could be drawn. No information on purity was provided.]

Prival et al. (1984) reported negative results for CI Direct Blue 218 (commercial samples) [the Working Group noted that no information on purity was provided] in the TA98 strain in the presence of metabolic activation (hamster liver S9), with and without modifications to promote the reduction of the azo bond (Prival & Mitchell, 1982). These modifications consisted of a 30-minute preincubation without agitation (to reduce oxygen) and the addition of flavin mononucleotide. *ortho*-Dianisidine (3,3′-dimethoxybenzidine), the parental aromatic amine of CI Direct Blue 218, was tested for mutagenicity

Table 4.1 Genetic and related effects of CI Direct Blue 218 in non-human mammals in vitro

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Gene mutation, *Tk* locus	Mouse, lymphoma L5178Y/*Tk*$^{+/-}$	–	+	40 µg/mL	Purity, NR	Mitchell et al. (1997)
Chromosomal aberrations	Chinese hamster, ovary (CHO) cells	–	–	500 µg/mL	Purity, NR	NTP (2020)
Sister-chromatid exchange	Chinese hamster, ovary (CHO) cells	+	–	200 µg/mL	Purity, NR	NTP (2020)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; *Tk*, thymidine kinase.
[a] +, positive; –, negative.

Table 4.2 Genetic and related effects of CI Direct Blue 218 in non-mammalian experimental systems

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Drosophila melanogaster	Sex-linked recessive mutation	–	NA	10 000 µg/mL (feed);1000 µg/mL (injected)	Purity, NR; single concentrations	Woodruff et al. (1985)
Salmonella typhimurium TA98, TA100	Reverse mutation	NT	–	1000 µg/plate	Purity, NR; tests under reductive conditions with and without riboflavin	Gregory et al. (1981)
Salmonella typhimurium TA98	Reverse mutation	NT	–	1000 µg/plate	Purity, NR; S9 10%; tests under reductive conditions with sodium dithionate	Gregory et al. (1981)
Salmonella typhimurium TA100	Reverse mutation	NT	Inconclusive	1000 µg/plate	Purity, NR; S9 10%; tests under reductive conditions with sodium dithionate	Gregory et al. (1981)
Salmonella typhimurium TA98, TA100, TA1537	Reverse mutation	NT	–	1091.90 µg/plate	Purity, NR; S9 10%; tests under reductive conditions with and without flavin mononucleotide	Prival et al. (1984)
Salmonella typhimurium TA1538	Reverse mutation	NT	–	545.9 µg/plate	Purity, NR; S9 30%; tests under reductive conditions in two protocols; (a) with and without rat caecal bacteria and (b) with flavin mononucleotide and S9 30%	Reid et al. (1984)
Salmonella typhimurium TA1535, TA1537, TA97, TA98, TA100	Reverse mutation	–	–	10 000 µg/plate	Purity, 44.8%; S9 10%; tests under oxidative conditions	Mortelmans et al. (1986)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NA, not applicable; NR, not reported; NT, not tested; S9, 9000 × *g* supernatant.
[a] –, negative.

under the same conditions and provided a clear positive response that was unchanged by the addition of CI Direct Blue 218. [The Working Group noted that this could indicate that the dye did not release *ortho*-dianisidine (3,3′-dimethoxybenzidine) after incubation with S9, because if this was the case, the test with the dye would provide a mutagenic response. In fact, the cleavage of the azo bond of CI Direct Blue 218 would generate 3,3′-dihydroxybenzidine, which gives negative results when tested with S9 at 10%. 3,3′-Dihydroxybenzidine is only mutagenic when S9 is present at 30% (NTP, 2018).] Prival et al. (1984) also reported negative results for CI Direct Blue 218 in strains TA100 and TA1537, and confirmed the negative results for TA98 using rat liver S9.

Reid et al. (1984) reported negative results for a commercial sample of CI Direct Blue 218 in strain TA1538 with endogenous metabolic activation. They tested the dye with and without incubation with a washed suspension of rat caecal bacteria (caecal reduction system) to promote reduction of the azo bond, simulating intestinal metabolism. The assay was also performed with flavin mononucleotide and Syrian golden hamster S9 using the protocol described by Prival & Mitchell (1982). [The Working Group noted that no information on purity was provided.]

Mortelmans et al. (1986) reported negative results for CI Direct Blue 218 (purity, 44.8%) in strains TA1535, TA1537, TA97, TA98, and TA100, with and without endogenous metabolic activation and preincubation. [The Working Group noted that the test was performed under oxidative conditions.]

[The Working Group noted that although the available *Salmonella* tests were performed with the five strains recommended by the Organisation for Economic Co-operation and Development (OECD, 2020), CI Direct Blue 218 was not tested in one of the strains sensitive to aromatic amines, e.g. YG1041 (Hagiwara et al., 1993). Zwarg et al. (2018) and Umbuzeiro et al. (2021) have shown the importance of including this strain to detect the mutagenicity of azo dyes containing NH_2 radicals, which is the case with CI Direct Blue 218.]

4.2.2 Causes immortalization

Matthews et al. (1993) reported equivocal results for commercial CI Direct Blue 218 in a study with the objective of introducing an improved method for detecting chemically induced morphological transformation of A31-1-13 BALB/c-3T3 cells. CI Direct Blue 218 was tested at six concentrations (125–2000 µM). No concentration–response relationship was observed, but at 250 and 500 µM, significant differences were observed in comparison with the negative control. [The Working Group noted that no information on purity was provided.]

4.3 Data relevant to comparisons across agents and end-points

CI Direct Blue 218 was not tested in biochemical and cell-based assays run by the United States Environmental Protection Agency (US EPA) and the United States National Institutes of Health Toxicity Forecaster/Toxicology in the 21st Century (ToxCast/Tox21) high-throughput screening programmes (Chiu et al., 2018; Guyton et al., 2018); see the monograph on gentian violet and leucogentian violet in the present volume for more details.

5. Summary of Data Reported

5.1 Exposure characterization

Colour Index (CI) Direct Blue 218 is a copper-chelated dimethoxybenzidine-based azo dye. It is used as a dye for cellulose, acetate, nylon, silk, wool, tissue and fine papers, and textile goods with a urea-formaldehyde finish.

Only one environmental study on CI Direct Blue 218 was identified, and this did not detect the dye in river water, sediments, or fish downstream of textile mills in a Canadian river. In general, dyes and pigments derived from benzidine and its congeners may be released into environmental waters as a constituent of industrial process waters, into the atmosphere by industrial sources, or via the disposal of dyed articles. Azo dyes are persistent bioaccumulative substances and, therefore, may contaminate groundwater, agricultural fields, aquatic plants, and fish.

The potential for occupational exposure to CI Direct Blue 218 occurs during the manufacturing process, from the application of the dye on products, and from further processing of dyed products that results in particle formation. Potential exposure routes include inhalation of dust or mist, accidental ingestion, and direct contact with the skin. No data on occupational exposure levels were identified.

No data on exposure to CI Direct Blue 218 in the general population have been identified; however, most environmental exposure to benzidine-based dyes has been described to be through contact with contaminated air, water, or soil. Benzidine-based dyes in finished products are not considered to migrate as a result of washing, or via perspiration or saliva.

Few specific regulations or guidelines for CI Direct Blue 218 exist, but there are guidelines for dimethoxybenzidine-based azo dyes and for azo dyes that are known to be metabolized to aromatic amines.

5.2 Cancer in humans

No data were available to the Working Group.

5.3 Cancer in experimental animals

Exposure to CI Direct Blue 218 caused an increase in the incidence of malignant neoplasms in both sexes of a single species (mouse) in a study that complied with Good Laboratory Practice (GLP), and an increase in the incidence of an appropriate combination of benign and malignant neoplasms in one sex of another species in a study that complied with GLP.

In $B6C3F_1$ mice exposed to CI Direct Blue 218 in the feed, there was a significant positive trend and significant increase in the incidence of hepatocellular carcinoma in males, and a significant positive trend in the incidence of hepatocellular carcinoma in females. In another species (rat), CI Direct Blue 218 in the feed significantly increased (with a positive trend and by pairwise comparisons) the incidence of squamous cell papilloma or carcinoma (combined) of the oral epithelium (pharynx) in F344/N male rats in a study that complied with GLP.

5.4 Mechanistic evidence

No data on absorption, distribution, metabolism, or excretion were available.

Few mechanistic data were available. CI Direct Blue 218 was mutagenic in one study in non-human mammals in a test in vitro, the mouse lymphoma assay, in the presence of metabolic activation. In Chinese hamster ovary cells, CI Direct Blue 218 induced sister-chromatid exchange only in the absence of metabolic activation but did not induce chromosomal aberrations in the presence or absence of metabolic activation. CI Direct Blue 218 was not mutagenic in *Drosophila melanogaster* and gave negative results in the available Ames tests, which did not cover a wide range of doses and strains.

No data relevant to the key characteristics of carcinogens were available from the Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes in the USA

because CI Direct Blue 218 was not tested in this assay battery.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of CI Direct Blue 218.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of CI Direct Blue 218.

6.3 Mechanistic evidence

There is *inadequate mechanistic evidence*.

6.4 Overall evaluation

CI Direct Blue 218 is *possibly carcinogenic to humans (Group 2B)*.

6.5 Rationale

The *Group 2B* evaluation for CI Direct Blue 218 is based on *sufficient evidence* for cancer in experimental animals. The evidence regarding cancer in humans is *inadequate* as no studies were available. The mechanistic evidence is *inadequate* for CI Direct Blue 218. The *sufficient evidence* for cancer in experimental animals is based on an increase in the incidence of malignant neoplasms in males and females of a single species in a study that complies with GLP, and an increase in the incidence of an appropriate combination of benign and malignant neoplasms in males of another species in another study that complies with GLP.

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