Substance Name: Disodium 3,3'-[[1,1'-biphenyl]-4,4'-diylbis(azo)]bis(4-aminonaphthalene-1-sulphonate) (C.I. Direct Red 28)

EC Number: 209-358-4

CAS Number: 573-58-0

SUPPORT DOCUMENT FOR IDENTIFICATION OF

DISODIUM 3,3'-[[1,1'-BIPHENYL]-4,4'-DIYLBIS(AZO)]BIS(4-AMINONAPHTHALENE-1-SULPHONATE) (C.I. DIRECT RED 28)

AS A SUBSTANCE OF VERY HIGH CONCERN BECAUSE OF ITS CMR[1] PROPERTIES

[1] CMR means carcinogenic, mutagenic or toxic for reproduction

CONTENTS

TABLES

Substance Name(s): Disodium 3,3'-[[1,1'-biphenyl]-4,4'-diylbis(azo)]bis(4-aminonaphthalene-1-sulphonate) (C.I. Direct Red 28)

EC Number(s): 209-358-4

CAS number(s): 573-58-0

The substance is identified as substance meeting the criteria of Article 57 (a) of Regulation (EC) 1907/2006 (REACH) owing to its classification as carcinogen category 1B.

Summary of how the substance meets the criteria set out in Article 57 (a) of REACH (Carcinogen 1B)

Disodium 3,3'-[[1,1'-biphenyl]-4,4'-diylbis(azo)]bis(4-aminonaphthalene-1-sulphonate) (C.I. Direct Red 28) is listed as Index number 611-027-00-8 in Regulation (EC) No 1272/2008[2] and classified in Annex VI, part 3, Table 3.1 (list of harmonised classification and labelling of hazardous substances) as carcinogen, Carc. 1B (H350: May cause cancer).

Therefore, this classification of C.I. Direct Red 28 in Regulation (EC) No 1272/2008 shows that the substance meets the criteria for classification as carcinogen in accordance with Article 57(a) of REACH.

Registration dossiers submitted for the substance:
The substance is not registered within REACH.

[2] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

JUSTIFICATION

1 Identity of the substance and physical and chemical properties

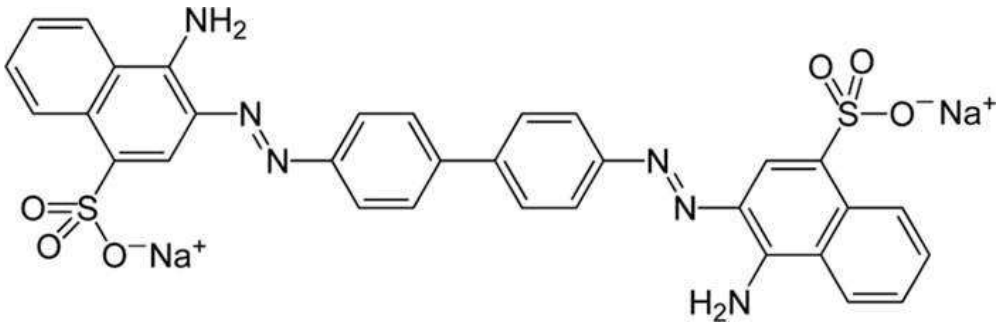
1.1 Name and other identifiers of the substance

Table 1: **Substance identity**

EC number:	209-358-4
EC name:	C.I. Direct Red 28; disodium 3,3'-[[1,1'-biphenyl]-4,4'-diylbis(azo)]bis(4-aminonaphthalene-1-sulphonate)
CAS number (in the EC inventory):	573-58-0
CAS number:	573-58-0 (Deleted CAS numbers: 550-08-3, 70248-72-5, 87440-95-7, 1000818-40-5)
CAS name:	1-Naphthalenesulfonic acid, 3,3'-[[1,1'-biphenyl]-4,4'-diylbis(2,1-diazenediyl)]bis[4-amino-, sodium salt (1:2)
IUPAC name:	Disodium 4-amino-3-[(4-{4-[(1-amino-4-sulfonatonaphthalen-2-yl)diazenyl]phenyl}phenyl)diazenyl]naphthalene-1-sulfonate
Index number in Annex VI of the CLP Regulation	611-027-00-8
Molecular formula:	$C_{32}H_{24}N_6O_6S_2.2Na$
Molecular weight range:	696.68 g/mol

Synonyms:	1-Naphthalenesulfonic acid, 3,3'-((1,1'-biphenyl)-4,4'-diylbis(azo))bis(4-amino-, disodium salt AI3-63036 Atlantic Congo Red Atul Congo Red Azocard Red Congo Benzo Congo Red Brasilamina congo 4B C.I. 22120 C.I. Direct Red 28 C.I. Direct Red 28, disodium salt Cerven kongo Cerven prima 28 Congazone sodium Congo Red Congo Red 4B Congo Red 4BX Congo Red CR Congo Red H Congo Red ICI Congo Red L Congo Red M Congo Red N Congo Red R Congo Red W Congo Red WS Cotton Red 4BC Cotton Red 5B Cotton Red L Diacotton Congo Red Direct Red 28 Direct Red C Direct Red DC-CF Direct Red K Erie Congo 4B Haemomedical Haemonorm Hemorrhagyl Hispamin congo 4B Kayaku Congo Red Mitsui Congo Red NSC 56651 NSC 7232 Peeramine Congo Red Red K Sodium diphenyldiazo-bis(alpha-naphthylaminesulfonate) Solucongo Sugai Congo Red Tertrodirect Red C Trisulfon Congo Red UNII-3U05FHG59S Vondacel Red CL

Structural formula:



1.2 Composition of the substance

Name: C.I. Direct Red 28; disodium 3,3'-[[1,1'-biphenyl]-4,4'-diylbis(azo)]bis(4-aminonaphthalene-1-sulphonate)

Description: benzidine-based dye

Substance type: mono-constituent

Table 2: Constituents

Constituents	Typical concentration	Concentration range	Remarks
Disodium 3,3'-[[1,1'-biphenyl]-4,4'-diylbis(azo)]bis(4-aminonaphthalene-1-sulphonate) 209-358-4	≥80%		

1.3 Physico-chemical properties

Table 3: Overview of physicochemical properties

Property	Value	Remarks
Physical state at 20°C and 101.3 kPa	Brownish-red powder; in water yellowish-red; in in ethanol orange	Source: HSDB
Melting/freezing point	>360 °C	Source: ChemIDPlus, experimental
Boiling point	n.a.	
Vapour pressure	2.24 x 10^{-30} mm Hg	Source: ChemIDPlus + SRC PhysProp Database, estimated
Water solubility	1.16 x 10^{5} mg/L	Source: ChemIDPlus + SRC PhysProp Database, experimental
Partition coefficient n-octanol/water (log value)	2.630	Source: ChemIDPlus + SRC PhysProp Database, estimated
Dissociation constant	n.a.	

n.a. = not available

2 Harmonised classification and labelling

C.I. Direct Red 28 is listed as Index number 611-027-00-8 in Regulation (EC) No 1272/2008 and classified in Annex VI, part 3, Table 3.1 as follows.

Table 4: Harmonised classification according to Annex VI, Part 3, Table 3.1 of Regulation (EC) No 1272/2008

Index No	**Classification**		**Labelling**		
	Hazard Class and Category Code[1]	**Hazard statement Code[2]**	**Pictogram, Signal Word Code**	**Hazard statement Code[2]**	**Notes**
611-027-00-8	Carc. 1B Repr. 2	H350 H361d ***	GHS08 Dgr	H350 H361d ***	

[1] Hazard Class and Category Code:

Carc. 1B:	Carcinogenic Category 1B
Repr. 2:	Toxic to Reproduction Category 2

[2] Hazard statement Code:

H350:	May cause cancer
H361d:	Suspected of damaging the unborn child
***:	In order not to lose information from the harmonised classifications for fertility and developmental effects under Directive 67/548/EEC, the classifications have been translated only for those effects classified under that Directive.

3 Environmental fate properties

Not relevant for the identification of the substance as SVHC in accordance with Article 57a.

4 Human health hazard assessment

See section 2 on harmonised classification and labelling.

5 Environmental hazard assessment

Not relevant for the identification of the substance as SVHC in accordance with Article 57a.

6 Conclusions on the SVHC Properties

6.1 CMR assessment

Disodium 3,3'-[[1,1'-biphenyl]-4,4'-diylbis(azo)]bis(4-aminonaphthalene-1-sulphonate) (C.I. Direct Red 28) is listed as Index number 611-027-00-8 in Regulation (EC) No 1272/2008[3] and classified in Annex VI, part 3, Table 3.1 (list of harmonised classification and labelling of hazardous substances) as carcinogen, Carc. 1B (H350: May cause cancer). Therefore, this classification of C.I. Direct Red 28 in Regulation (EC) No 1272/2008 shows that the substance meets the criteria for classification as carcinogen in accordance with Article 57(a) of REACH.

[3] Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

ANNEX I. SUPPLEMENTARY INFORMATION ON HUMAN HEALTH EFFECTS

I.1. Toxicokinetics (absorption, metabolism, distribution and elimination)

The azo linkage is the most labile portion of an azodye molecule and may easily undergo enzymatic breakdown in mammalian organisms, including man. The azo linkage may be reduced and cleaved, resulting in the splitting of the molecule in two parts (Brown & DeVito, 1993, as cited in Øllgaard *et al*., 1998). In case of Direct Red 28, reduction and cleavage of the two azo linkage sites results in the formation of benzidine (see figure I.1 and I.2)

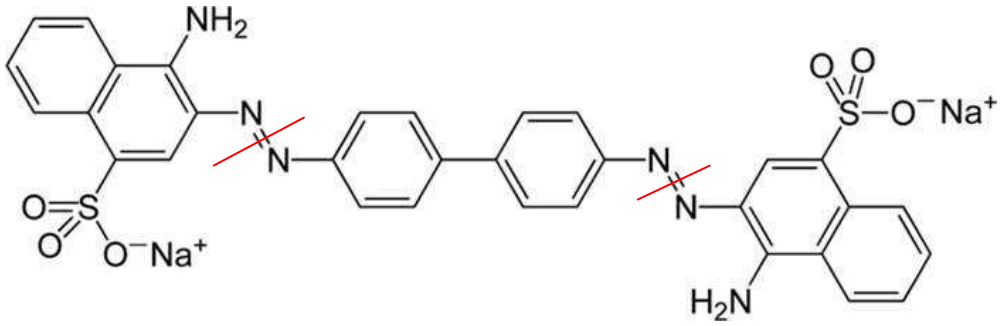


Figure I.1. Cleavage sites at the azo linkages in case of azo reduction in Direct Red 28, indicated with red lines.

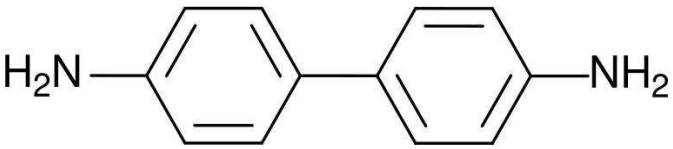


Figure I.2. Structure of benzidine

The anaerobic environment of the lower gastrointestinal tract of mammals is well suited for azo reduction. Several anaerobic intestinal bacteria are capable of reducing the azo linkage. The majority of these bacteria belong to the genera *Clostridium* and *Eubacterium*. They contain an enzyme associated

with the cytochrome P450, also termed azo-reductase. It is a non-specific enzyme, found in various micro-organisms and in all tested mammals (NIOSH, 1980, as cited in Øllgaard *et al*., 1998).

In mammalian organisms azo-reductases are, with different activities, present in various organs like liver, kidney, lung, heart, brain, spleen and muscle tissues. The azo-reductase of the liver, followed by the azo-reductase of the kidneys possesses the greatest enzymatic activity. (Øllgaard *et al*., 1998)

Benzidine and its congeners have been found in the urine of humans and animals exposed to azodyes (Lowry *et al*., 1980, and Lynn *et al*., 1980, as cited in Reid *et al*.,

1984a; Robens *et al*., 1980)

I.2. Mutagenicity

I.2.1. *In vitro* data

Table I.1. Ames test data for Direct Red 28 (obtained from the CCRIS database).

Bacterial strain	S9	method	dose	Result	reference
TA98	+, hamster	Preincubation	0-300 nmol/plate	+	1
TA98	+, hamster	Preincubation	0.1-0.5 µmol/plate	+	2
TA1538	+, rat	Standard plate	0.1-1 µmol/plate	-	3
TA1538	+, hamster	Standard plate	0.1-1 µmol/plate	-	3
TA98	-	Standard plate	1-500 µg/plate	-	4
TA98	+, rat	Standard plate	1-500 µg/plate	-	4
TA100	-	Standard plate	1-500 µg/plate	+	4
TA100	+, rat	Standard plate	1-500 µg/plate	-	4
TA98	+, rat	preincubation	20-2,500 µg/plate	-	5
TA1538	+, rat	preincubation	20-2,500 µg/plate	-	5

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2. Zhou, Y., You, X., and Ye, X. (1987) Mutagenicity of benzidines and their congener derivative dyes. Huanjing kexue 8(2), p.31-34.
3. Reid, T.M., Morton, K.C., Wang, C.Y., and King C.M. (1984) Mutagenicity of azo dyes following metabolism by different reductive/oxidative systems. Environ. Mutagen. 6(5), p.705-717.
4. Kaur, A., Sandhu, R.S., and Grover, I.S. (1993) Screening of azo dyes for mutagenicity with Ames/Salmonella assay. Environ. Mol. Mutagen. 22(3), p. 188-190.
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The Ames test data in table I.1 show mostly negative results, with remarkable positive outcomes in TA98 when hamster S9 was used. Robertson *et al*. (1982) and Reid *et al*., (1983) have shown that the standard Ames test needs some adaptations to detect the mutagenicity of Congo Red, as two metabolic steps are required: azo reduction to form benzidine, and subsequent oxidation. The azo reduction normally does not occur in the Ames test, but could be achieved by the addition of flavin-cofactors (Robertson *et al*., 1982) or pre-incubation with whole-cell rat cecal bacteria (Reid *et al*., 1983). This explains the mostly negative results in the standard Ames tests. The necessary oxidation step is probably the addition of an OH-group (Reid *et al*., 1984b). *N*-acetylation of benzidine makes the compound more potent in mutagenicity (Reid *et al*., 1984b).

Kombrust and Barfknecht (1984) have found that Congo Red and its azo reduction product, benzidine, were more potent inducers of DNA repair in hamster than in rat hepatocytes, indicating hamsters may have more of the required metabolic enzymes in their liver for producing the mutagenic metabolites of Congo Red. This explains the positive results in the Ames test with hamster S9 in TA98.

I.2.2. *In vivo* data

The adduct isolated from liver DNA of rats injected with benzidine or N-acetylbenzidine (ABZ) has been identified as *N-dG-N'* –ABZ. The same adduct was found in rats dosed with Direct Red 28 intraperitoneally, while it had been found earlier that Congo Red did not undergo rat hepatic azo reduction to benzidine *in vitro* and *in vivo*. It was postulated that this indicates that Congo Red binds to rat liver DNA following azo reduction to benzidine by gut micro flora and subsequent N-acetylation in the liver to N-acetylbenzidine. (Kennelly *et al*., 1984).

I.2.3. Summary and discussion of mutagenicity

The available Ames test data on Direct Red 28 are conflicting, but an *in vivo* rat study shows that administration of Direct Red 28 leads to DNA adducts of the N-acetylated benzidine-metabolite. This metabolism to benzidine seems to take place in the gut and not in the liver in the case of Direct Red 28. The benzidine is then N-acetylated in the liver to N-acetylbenzidine, which can form a DNA-adduct. As also shown by Robertson *et al.* (1982), the lack of azo reduction in the standard Ames test explains the mostly negative outcome of Direct Red in Ames test even in presence of S9, while benzidine gives mostly positive results in Ames tests (source: CCRIS database). S9 is a liver homogenate and rat S9 apparently does not provide the metabolic activity necessary for azo reduction of Direct Red 28 to benzidine, while hamster S9 does seems to provide this.

As benzidine has been found in the urine of azodye exposed humans and as azo-reductase has been found in the gut of all tested mammals, it may be expected that Direct Red 28 is metabolized to a mutagenic substance in humans. Therefore, Direct Red 28 should be considered mutagenic.

I.3. Carcinogenicity

I.3.1. Carcinogenicity: oral

Direct Black 38, Direct Blue 6 and Direct Brown 95 were found to be hepato-carcinogenic to rats during a 13-week oral subchronic toxicity trial (Robens *et al.*, 1980). These are benzidine-based dyes just like Direct Red 28. The benzidine metabolite of Direct Red 28 has been classified as *carcinogenic to humans* (group 1) by IARC and as Carc 1A by harmonized classification.

I.3.2. Human information

Epidemiological studies on cancer incidences in people working with azodyes showed increased bladder cancer incidences in azodye exposed workers (e.g. Case *et al*., 1954; Gonzales *et al*., 1988; Golka *et al.,* 2008).

I.3.3. Summary and discussion of carcinogenicity

Direct Red 28 has been classified as Carc 1B (H350) (Classification Index number 611-027-00-8).

I.4. Toxicity for reproduction

I.4.1. Developmental toxicity

I.4.1.1. Non-human information

In one study, Beaudoin *et al.* (1964) investigated the teratogenicity of Direct Red 28 in Wistar rats, with the females receiving a single intraperitoneal injection of a 2% aqueous solution of 140, 200 or 400 mg Direct Red 28 per kg bodyweight on gestation day 8. Teratogenicity was seen at 20 mg/100 g, with 15.4% of the survivors malformed. The top dose of 40 mg/100 g was found to be lethal.

In the study of Gray *et al*. (1992) male and female mice were exposed in utero to the diazodye Congo red (CR). Maternal CR treatment inhibited testicular and ovarian function in the offspring after oral administration of 1 or 0.5 g/kg bw/day on gestational day 8-12. The testes of male offspring from CR exposed dams were small in size and contained hypospermatogenic seminiferous tubules. However, despite the fact that testis weight was reduced by more than 70% in some males, they displayed normal levels of fertility when mated to untreated females for over 10 months. In contrast, female offspring from CR exposed dams produced only about half as many litters and pups as the control pairs did under long term mating conditions. Histological examination of the ovaries revealed that subfertility was correlated with ovarian atrophy. Females lacking maturing follicles were considerably less productive (1.3 litters and 11.5 pups) than treated females with histologically normal ovaries (7.1 litters and 78.1 pups). It was concluded that prenatal exposure to the dye CR affects the gonads of both male and female offspring, but only the female offspring display reduced fertility. (Gray *et al*., 1992)

In another study, the effects of prenatal exposure to azodyes on testicular development were studied in mice. Pregnant CD-l mice were administered 0 or l mg azodye/kg bw orally on gestational day eight to 12 and were observed for clinical signs of toxicity. Maternal body weight, neonatal viability, and growth of the offspring were monitored. The male offspring were /sacrificed/ when 45 to 50 days old and the testes, seminal vesicles, and caudal epididymides were removed and weighed. The testes were examined for histopathological changes and sperm counts were recorded. All dyes significantly decreased maternal body weight gain but none affected neonatal viability or growth of the pups. Congo red, direct black 38 and direct blue 6 significantly decreased testicular weight and induced seminiferous tubule atrophy. Many of the tubules contained no germ cells. Azodyes derived from benzidine given the progonadal stage of organogenesis induce testicular toxicity in male offspring. The chemical structure requirements for gonadal developmental toxicity are that the dye must contain the benzidine moiety. Dimethyl or dimethoxy substitution eliminates developmental toxicity. (Gray *et al*., 1993)

I.4.2 Summary and discussion of reproductive toxicity

Direct Red 28 has been classified as Repro 2 (H361d) (Classification Index number 611-027-00-8).

I.5 References

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