Annex XV SVHC dossier

PROPOSAL FOR IDENTIFICATION OF A SUBSTANCE AS A CMR 1A OR 1B, PBT, vPvB OR A SUBSTANCE OF AN EQUIVALENT LEVEL OF CONCERN

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

Submitted by: The Netherlands

Submission date: 5 August 2013

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EC Number(s): 209-358-4

CAS number(s): 573-58-0

The substance is proposed to be 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[1] 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.

[1] 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.

PART I

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

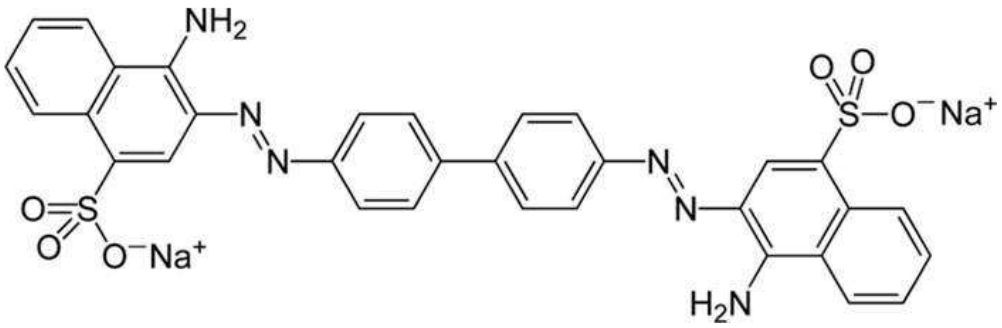
1.1 Name and other identifiers of the substance

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	2.24×10^{-30} mm Hg	Source: ChemIDPlus + SRC PhysProp Database, estimated
Water solubility	1.16×10^{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[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.

[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.

PART II

INFORMATION ON USE, EXPOSURE, ALTERNATIVES AND RISKS

INFORMATION ON MANUFACTURE, IMPORT/EXPORT AND USES –CONCLUSIONS ON EXPOSURE

MANUFACTURE, IMPORT/EXPORT

There is no registration of C.I. Direct Red 28 (Direct Red 28) within REACH, while the deadline for registration of CMR substances > 1 tonne/year has passed. This indicates the substance is not manufactured or imported in the EU at levels > 1 tonne/year/company. The four contacted notifiers for Direct Red 28 within the C&L Inventory offer Direct Red 28 on their webpages, mostly in quantities of 1-100 g, but with options for bulk orders. They were contacted with the question what quantities they could deliver, and what quantities had been delivered in Europe the past years. Two notifiers responded, where one indicated it could deliver maximally 500 kg, but the maximum quantity delivered in Europe was 60 kg. This notifier stated not to manufacture the substance itself, but to trade it. The other responding notifier indicated to be able to maximally deliver 25 kg, that the notifier had also delivered such quantity in Europe and that this quantity was delivered to production companies, although it remains unknown what type of production companies. This notifier also did not manufacture the substance, but traded it. A third notifier only indicated the minimum quantity that could be ordered was 1 kg.
An internet search showed there are many suppliers of Direct Red 28 outside of the EU, especially in China (e.g. see the list of suppliers of Chemexper.com:
http://www.chemexper.com/chemicals/supplier/cas/573-58-0.html)

From this (scarce) information, there is no indication that Direct Red 28 is manufactured in the EU at all. However, it may be estimated that at least (60 + 25 =) around 85 kg is delivered by half of the notifiers, thus roughly > 100 kg per year is delivered to European customers.

No information on import or export of the substance or products containing the substance, could be found.

USES

The major use of Direct Red 28 is as a dye for e.g. textile and paper (Green, 1990). The Encyclopedia Brittanica states, however, that Congo Red “has been superseded by dyes more resistant to light and to washing”. The use in textiles that come into contact with the skin is prohibited in the EU since 2002 (European Directive 2002/61/EC, now included in EC 1907/2006 (REACH regulation), see below).

Direct Red is also used as a pH indicator, as an addition to culture media and for biological staining, e.g. in histology (Budavari, 1996; Dapson, 2009; Encyclopedia Brittanica), as well as a gelling agent for poly(vinyl alcohol) in applications where coloured films or coatings can be tolerated (Kirk-Othmer Encyclopedia of Chemical Technology, 1983).

C.I. Direct Red 28 is a benzidine-based dye; such substances are known to be cleaved metabolically to benzidine, a carcinogenic substance (Carc 1A by harmonized classification). The presence of azodyes that release one or more of specified (in Appendix 10 of REACH) 22 aromatic amines (including benzidine) at levels > 30 mg/kg in textile and leather articles (or the dyed parts thereof) which may come into direct and prolonged contact with the human skin or oral cavity is restricted in the EU (EC 1907/2006 (REACH regulation), Annex VII, entry 43) since 2006. Even before that, it was restricted under European Directive 2002/61/EC. In the Netherlands, this restriction started in 1998 ("Warenwetbesluit azo-kleurstoffen"). Data obtained from the Dutch Food and Product Safety Authority (NVWA; see table 5) show that even after 1998, aromatic amines have been detected in textile and leather consumer articles, indicating azodyes are still used. Additionally, nearly all articles for which RAPEX alerts were issued in 2009-2012 because of too high levels of benzidine, came from China or India (table 6), This underpins the general expectation that azodyes are mostly used in countries outside the EU (e.g. Dapson, 2009), from where products are then imported into the EU.
A study of the Danish EPA on the occurrence of chemicals in tattoo inks in 2012 showed no detectable (detection limit was 2 µg/g) levels of benzidine in the analysed tattoo inks (D-EPA, 2012). In 2005, this agency also did not find aromatic amines in textile colourants for hobby use (detection limit 30 mg/kg; D-EPA, 2005). The German Stiftung Warentest found an azodye in a bath duck in a test of toys in 2002 (without specifying which azodye), indicating these substances are not only used in the textiles for which there is already a restriction.

All these reports on the detection of azodyes in consumer products indicate a limited continued use of such substances, in products used throughout Europe, which therefore may be assumed for Direct Red 28, too.

Table 5: Overview of results of analyses of consumer products for the aromatic amines indicative of azodye presence over the last 18 years (kindly provided by the Dutch Food and Product Safety Authority (NVWA)).

Year of study	Type of products analyzed	Country of sampling	No. of products	No. of products with aromatic amines	% of products with aromatic amines	No. of products with benzidine	% of products with benzidine	Maximum detected level of benzidine (mg/kg)	Ref.[2]
1995	Clothing	NL	132	6	5%	n.a.	n.a.	n.a.	KvW, 1995
1996	Clothing	NL	200	15	8%	n.a.	n.a.	n.a.	KvW, 1996
1997	Clothing	NL	116	13	11%	n.a.	n.a.	n.a.	KvW, 1997
1999	Texile and leather products, toys	NL	160	4	3%	n.a.	n.a.	n.a.	Consumentenbond, 1999
2000	Clothing	NL	141	n.a.	n.a.	n.a.	n.a.	n.a.	KvW, 2000
2001	Leather clothing and other leather products	NL	70	9	13%	n.a.	n.a.	n.a.	KvW, 2001
2003	Leather working gloves	NL	31	6	19%	n.a.	n.a.	< 30	VWA, 2003
2003	Leather working gloves	NL	109	17	16%	n.a.	n.a.	1403	VWA, 2004a
2004	Toys with textile and leather, that are expected to be mouthed	NL	54	4	7%	n.a.	n.a.	< 30	VWA, 2004b

2004	Textile and (artificial) leather consumer products, e.g. wallets, bags, belts, gloves and watchstraps	NL	65	12	18%	n.a.	n.a.	1857	VWA, 2004c
2004	Textile products that come into contact with the skin, mainly brightly coloured ones	NL	341	11	3%	n.a.	n.a.	47	VWA, 2005a
2005	Underwear	NL	83	10	12%	1	1,2%	21	VWA, 2005b
2005	Clothing from third world shops	NL	59	23	39%	17	28,8%	2746	VWA, 2005c
2006	Textile products from army and navy dump shops	NL	99	8	8%	5	5,1%	552	VWA, 2007a
2006	Textile (and leather) articles from big chins of stores with cheap articles + 1 expensive bed store	AT	48	0[1]	0%[1]	0	0,0%	< 30	CLEEN, 2007
2006	Textile and leather articles, wide range, and 2 colouring agents	DK	40	0[1]	0%[1]	0	0,0%	< 30	CLEEN, 2007
2006	Textile and leather articles from companies importing cheap products, mainly from China	EE	17	0[1]	0%[1]	0	0,0%	< 30	CLEEN, 2007
2006	Textile and leather articles, mostly imported	GR	105	2[1]	2%[1]	n.a.	n.a.	n.a.	CLEEN, 2007
2006	Textile and leather articles from low-price stores or shops with direct import from Asia	NO	40	0[1]	0%[1]	0	0,0%	< 30	CLEEN, 2007
2006	Textile and leather articles, mostly from Poland itself	PL	30	1[1]	3%[1]	0	0,0%	< 30	CLEEN, 2007
2007	Sports clothing	NL	141	2	1%	2	1,4%	1515	VWA, 2007b
2008	Childrens clothing	NL	181	0	0%	n.a.	n.a.	n.a.	VWA, 2008
2009	Leather clothing	NL	44	2	5%	1	2,3%	2229	VWA, 2009
2011	Scarfs and jeans	NL	108	7	6%	5	4,6%	1170	VWA, 2011

n.a. = not available;

[1] = only levels >30 mg/kg legal threshold were considered

[2] References:CLEEN (Chemical Legislation European Enforcement Network), 2007, “Eurazos. Final report”.

Dutch Food and Product Safety Authority (Voedsel en Warenautoriteit, VWA), 2003, report ND03r015-02.

Dutch Food and Product Safety Authority (VWA), 2004a, “Handhavingsactie azo-kleurstoffen in werkhandschoenen [Enforcement action azodyes in working gloves]”, report ND03r015-03.

Dutch Food and Product Safety Authority (VWA), 2004b, “Handhavingsactie azo-kleurstoffen in speelgoed [Enforcement action azodyes in toys]”, report ND04o052-02.

Dutch Food and Product Safety Authority (VWA), 2004c, “Handhavingsactie azo-kleurstoffen in textiel en lederwaren [Enforcement action azodyes in textile and leather articles]”, report ND04o052-01.

Dutch Food and Product Safety Authority (VWA), 2005a, “Azo-kleurstoffen in textiel [Azodyes in textile]”, report ND04o052-03.

Dutch Food and Product Safety Authority (VWA), 2005b, “Azo-kleurstoffen in onderkleding [Azodyes in underwear]”, factsheet dated april 2006 (confidential).

Dutch Food and Product Safety Authority (VWA), 2005c, “Handhavingsactie azo-kleurstoffen in 3e wereldwinkel kleding [Enforcement action azodyes in 3d world shop clothing]”, report ND05o502.

Dutch Food and Product Safety Authority (VWA), 2007a, “Azocolourants in textile. Dutch contribution to the CLEEN project Eurazos”, factsheet dated February 2007.

Dutch Food and Product Safety Authority (VWA), 2007b, report ND071915.

Dutch Food and Product Safety Authority (VWA), 2008, report ND08191B.

Dutch Food and Product Safety Authority (VWA), 2009, report ND09191D-3.

Dutch Food and Product Safety Authority (VWA), 2011, report PHNT1106.
Dutch Food and Product Safety Authority (Keuringsdienst van Waren, KvW), 1995, report TX 12.
Dutch Food and Product Safety Authority (KvW), 1996, report TX 18.
Dutch Food and Product Safety Authority (KvW), 1997, report TX 19.
Dutch Food and Product Safety Authority (KvW), 2000, report ND TEX 002/01
Dutch Food and Product Safety Authority (KvW), 2001, report ND TEX 002/02
Dutch Union of Consumers [Consumentenbond], 1999, Consumentengids april 1999, p. 48 – 50.

Table 6: Cases where benzidine was found in consumer articles in the EU in 2009-2012 (RAPEX)

Year	Notifying country	Product	Country of origin	Benzidine level
2009	Germany	Baseball cap	China	598 mg/kg
2009	Finland	White scarf with print	China	180 mg/kg
2009	Finland	Green scarf	India	880 mg/kg
2009	Finland	Red scarf	India	880 mg/kg
2009	Finland	Red scarf	India	210 mg/kg
2009	Germany	Work gloves	Unknown	208 mg/kg
2009	Germany	Red silk scarf	China	124 mg/kg
2009	Germany	Black silk scarf	Unknown	617 mg/kg
2009	Germany	Black leather gloves	China	1.504 mg/kg
2009	Finland	Red-black scarf and blue-green scarf	India	44 and 240 mg/kg
2009	Finland	Red part of striped bedspread	China	740 mg/kg
2009	Finland	Dark blue scarf and orange scarf	India	590 mg/kg and 210 mg/kg
2009	Finland	Scarf with pattern	China	460 mg/kg
2009	Finland	Turqoise and blue colours in 2 scarves	India	100-230 mg/kg
2010	Germany	Pink silk scarf	India	130 mg/kg
2010	Denmark	Red scarf	India	196 mg/kg
2010	Finland	Scarf	India	660 mg/kg
2010	Germany	Black leather gloves	China	344 mg/kg
2010	Germany	Black leather purse	India	390 mg/kg
2010	Germany	Decorated floral scarf	India	96.7 mg/kg
2010	Finland	Grey scarf	India	100 mg/kg
2010	Germany	Red dress	China	88.9 mg/kg
2010	Finland	Red silk scarf and purple silk scarf	India	77 and 96 mg/kg
2010	Finland	Black scarf	China	400 mg/kg
2010	Finland	Navy blueT-shirt and red T-shirt	India	1100 and 910 mg/kg
2010	Germany	Black blouse	China	1571 mg/kg
2011	Finland	Black scarf	India	4500mg/kg

2011	Bulgaria	Sports shoes	China	52.1 mg/kg
2011	Germany	Jeans	China	48mg/kg
2011	Greece	Ladies dress	India	59 mg/kg
2011	The Netherlands	Black scarf	India	1170 mg/kg
2011	Germany	Towel	India	1 501 mg/kg
2011	Finland	Bed spreads and cushion cover	India	130-210 mg/kg
2011	Greece	Men black T-shirt	Syria	4118 mg/kg
2011	Cyprus	Blue children's T-shirt	China	100 mg/kg
2011	Germany	Black hat	China	189 mg/kg
2011	Spain	Denim dress	Bangladesh	1 183 ppm
2011	Cyprus	Black dress	Greece	500 mg/kg
2011	Hungary	Black scarf	India	3730 mg/kg
2011	Cyprus	Red scarf and blue scarf	India	Both 400 mg/kg
2011	Denmark	Green cardigan	China	1248,7 mg/kg
2011	Denmark	Children's sweatshirt	India	219,21 ppm
2012	Hungary	Leather bracelet	India	590 mg/kg
2012	Hungary	Men's leather wrist band	China	48.9 mg/kg
2012	Hungary	Black leather wristband	China	801.6 mg/kg
2012	Poland	Jeans	Germany	84.5 mg/kg

CURRENT KNOWLEDGE ON ALTERNATIVES

Alternatives for the dying of textile and paper

The Encyclopedia Brittanica states that Congo Red "has been superseded by dyes more resistant to light and to washing". The data in table 5 indicate that most textile and leather products, even brightly coloured ones, are produced without azodyes, implicating suitable alternatives are already available.

Direct Red 28 is used for its red colour. Inspection of websites of dye or chemical manufacturers on other red dyes delivered hundreds of possible red dyes, i.e. dyes containing "red" in their CI name. Some examples are given in table 7, while the "Direct dyes" and "Sudan dyes" were not provided in this table as they are benzidine-based dyes too. Therefore, they are not better alternatives health-wise. Of course, every application has its own demands on the chemical and physical properties of the dye, and colour hues will differ. It is unknown whether the listed examples of alternative red dyes are suitable for colouring textiles and paper, although for some, such use has been reported.

Table 7: Non-exhaustive list of dyes containing “red” in the CI name, as found on webpages of dye or chemical manufacturers.

Common name (if any)	**CI Name**	**CI Number**	**CAS**	**Hazard Statements**
	Disperse Red 1	11110	2872-52-8	H317
	Disperse Red 11	62015	2872-48-2	
	Disperse Red 19	11130	2734-52-3	
	Acid Red 1	18050	3734-67-6	
	Acid Red 14	14720		
Azo Red A		14910	5858-64-0	
Allura Red AC		16035	25956-17-6	
Emin Red A		16085		
	Acid Red 18	16255		
Amaranth, Red No. 2, E123	Acid Red 27	16185	915-67-3	H315-H319-H335
	Acid Red 52	45100		
	Acid Red 73	27290		
	Acid Red 87	45380		
	Acid Red 92	45410		
	Acid Red 94	45440		
	Acid Red 114	23635	6459-94-5	
	Basic Red 1	45160		
	Basic Red 2	50240		
	Solvent Red 18	21260		
	Solvent Red 24	26105		
	Solvent Red 27	26125		
	Solvent Red 49	45170:1		
	Solvent Red 52	68210		
	Solvent Red 111	60505		
	Solvent Red 135	564120		
	Solvent Red 140	~		
Orcein, archil, orchil, lacmus	Natural Red 28		1400-62-0	
Quinaldine red			117-92-0	

Alternatives for biological staining

Direct Red 28 (or Congo Red) is very popular for histopathological amyloid staining when amyloidosis is suspected in a patient, a condition wherein normally soluble proteins become insoluble and are deposited as aggregates in the extracellular space of organs and tissues. Alternatives for Congo Red staining are Crystal Violet, Methyl Violet, Thioflavin T or Thioflavin S. Whereas Congo Red is about 96% sensitive (i.e. giving 4% false negatives), Crystal Violet and Methyl Violet are only 70% sensitive. Crystal Violet and Methyl Violet may stain other tissue components and they require aqueous mounting media, disabling the possibility for permanently staining the slides for archiving. The thioflavins are fluorescent dyes, which require the availability of a fluorescence microscope with a FITC filter. They are 100% sensitive for amyloids, but detection can be hampered by autofluorescence of tissues in the same yellowish colour. As for the Violet stains, aqueous mounting media are necessary, thus permanent staining is not possible. (Wenk, 2010).

Alternatives for use as pH indicator

Direct Red 28 (or Congo Red) changes from blue to red at pH 3.0 to 5.2. An alternative can be anthocyanidin, a common plant pigment, which changes from red to pink at pH 3 to 4-5. Bromophenol Blue changes from yellow to purple between pH 3.0 and 4.6. Methyl yellow changes from red to yellow at pH 2.9 to 4.0. Methyl Orange changes from from red to yellow at pH 3.1 to 4.4. Methyl red changes from red to yellow at pH 4.4 to 6.2. (Wikipedia)

Conclusion on alternatives

There seem to be sufficient alternatives for the use for colouring textile and paper, as well as for the use as pH indicator. The alternatives for biological staining have some serious drawbacks, while the staining has an important social benefit in medical care. This use takes place in a laboratory by trained personnel.

RISK-RELATED INFORMATION

No EU risk assessments for the substance have been identified. In order to assess potential risks associated with this substance, information on physicochemical properties was used to derive estimates of potential worst case consumer exposure using ECETOC-TRA and Consexpo software.
This "indicative" risk characterisation then considered the exposure assessments and the DNEL or DMEL values obtained from the literature. No DNEL/DMEL values were found in the literature for Direct RED 28. Instead, a NSLR (No Significant Risk Level) from the California OEHHA Toxicity Criteria Database of 1.5×10^{-6} mg/kg bw/day (oral) was used as a basis for comparison with the exposure estimates.
The "indicative" worst-case exposure estimates derived using ECETOC-TRA and Consexpo software are:

- Clothing: 37 mg/kg/day (dermal) and 0.16 mg/kg/day (oral)
- Printed paper: 3.3×10^{-4} mg/kg/day (dermal plus oral)

It has not been possible to identify a risk characterisation ratio for the substance as no appropriate DNEL or DMEL (or equivalent) no/minimal effect value has been identified for the substance. However, based on comparison with similar substances, it is likely that the above exposure estimates will be higher than minimal effect levels.
However, the above should be treated with caution given the exposure assessments are based on screening level assessments, with mainly default values used in relation to the use of the substance

within the models concerned. This is because of the relative paucity of information on use of the substance.

Overall, taking a worst case screening level exposure assessment, it appears that there could be some potential for unacceptable risks related to the use of the substance in consumer products, but there is insufficient information to reach a firm conclusion on this. It should be noted that there is minimal information available on current uses in consumer products.

Furthermore, the exposure assessment approaches used are generally based on worst-case default assumptions in relevant models used in the context of REACH. There is considered to be significant potential to further refine the estimated levels of exposure, which would in turn provide additional information on the potential risks.

REFERENCES

Budavari, S. (ed.).(1996) The Merck Index - An Encyclopedia of Chemicals, Drugs, and Biologicals. Whitehouse Station, NJ: Merck and Co., Inc., p. 423.

ChemIDplus Lite, United States National Library of Medicine, http://chem.sis.nlm.nih.gov/chemidplus/ProxyServlet?objectHandle=Search&actionHandle=getAll3DMViewFiles&nextPage=jsp%2Fcommon%2FChemFull.jsp%3FcalledFrom%3Dlite&chemid=0000573580&formatType=_3D (accessed May 2013).

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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)

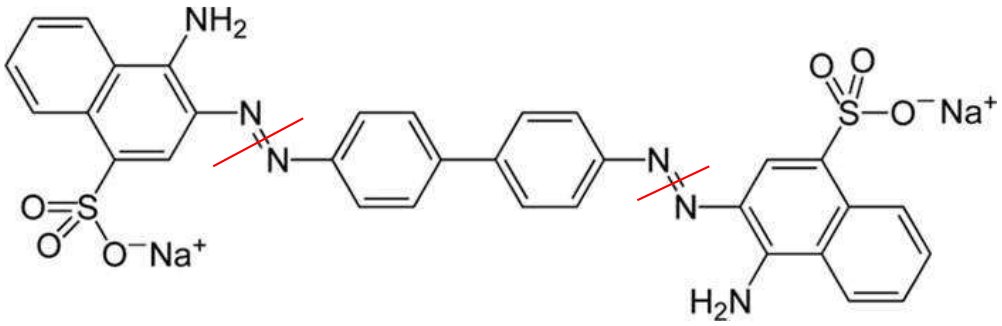

Figure I.1. Cleavage sites at the azo linkages in case of azo reduction in Direct Red 28, indicated with red lines.

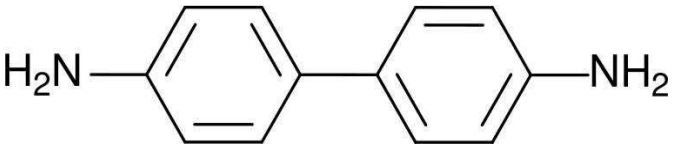

Figure I.2. Structure of benzidine

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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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).

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