Screening Assessment for the Challenge

2-Naphthalenol, 1-[[4-(phenylazo)phenyl]azo]-
Solvent Red 23
Chemical Abstracts Service Registry Number

85-86-9

Environment Canada
Health Canada

September 2011

Synopsis

Pursuant to section 74 of the *Canadian Environmental Protection Act (CEPA), 1999*, the Ministers of the Environment and of Health have conducted a screening assessment on 2-naphthalenol, 1-[[4-(phenylazo)phenyl]azo]-, (herein referred to as Solvent Red 23), Chemical Abstracts Service Registry Number[1] 85-86-9.

This substance was identified as a high priority for screening assessment and included in the Challenge initiative under the Chemicals Management Plan because it was found to meet the ecological categorization criteria for persistence, bioaccumulation potential and inherent toxicity to non-human organisms and is believed to be in commerce in Canada. The substance, Solvent Red 23, was not considered to be a high priority for assessment of potential risks to human health, based upon application of the simple exposure and hazard tools developed for categorization of substances on the Domestic Substances List.

Solvent Red 23 is an organic substance that is used in Canada primarily in oils, fats and waxes, but also in alcohol-based, ester and hydrocarbon solvents, in polystyrene, in cosmetics and as an indicator. It is also used as a pesticide colourant and has been known to be used as a textile dye. It is not naturally produced in the environment. Solvent Red 23 is not reported to be manufactured in Canada. In 2006, Solvent Red 23 was not reported to be imported or used in Canada. However, four companies reported importing between 100 and 1000 kg of the substance into the country in 2005 in products in response to Canada's Section 71 survey under CEPA 1999.

Based on reported use patterns in Canada related to personal care products, it is anticipated that 100% of products containing Solvent Red 23 could be released to sewer, surface water or land during their use. Sewer water is the medium potentially receiving the greatest proportion of Solvent Red 23 during product use. It is anticipated that the majority of Solvent Red 23, bound to sewage sludge from down-the-drain releases of cosmetics and personal care products to sewage treatment plants, will be sent entrained in sludge to landfills. In addition to being found in landfills, some of the biosolids from wastewater treatment facilities may be applied to land as a fertilizer or soil conditioner for uses in agriculture, forestry and reclamation and a small percentage may be incinerated.

Solvent Red 23 is not expected to be soluble in water or to be volatile, but is expected to adsorb to particles because of its hydrophobic nature. For these reasons, after release to water, Solvent Red 23 will likely be found in sediments and, possibly to a much lesser extent, in agricultural soil that has been amended with biosolids. Solvent Red 23 is not expected to be significantly present in other media and is not expected to be subject to long-range atmospheric transport.

Based on the physical and chemical properties of Solvent Red 23, it is expected to be persistent in soil, sediment, and water. However, experimental data relating to the bioaccumulation potential of two relatively close structural analogues suggests that this dye has a low potential to accumulate in the lipid tissues of organisms. This substance, therefore, meets the persistence criteria but does not meet the bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations*. In addition, experimental toxicity data for chemical analogues

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suggest that Solvent Red 23 does not cause acute harm to aquatic organisms exposed at low concentrations.

For this screening assessment, a conservative exposure scenario was selected in which a single wastewater treatment plant was assumed to discharge all the maximum quantity range of Solvent Red 23 based on the survey data from 2005. Additionally, since Solvent Red 23 may be used in consumer products, a conservative consumer release scenario was developed based on an estimate of the quantity of this dye in Canadian commerce. The predicted environmental concentration in water was below the predicted no-effect concentration calculated for sensitive aquatic species.

Based on the information available, it is concluded that Solvent Red 23 is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

Exposure of the general population to Solvent Red 23 from environmental media is expected to be negligible. However, exposure can occur through the use of cosmetics and personal care products containing Solvent Red 23. Empirical health effects data for Solvent Red 23 are limited. Solvent Red 23 is a member of a class of substances characterized by the presence of one or more azo (-N=N-) groups, which can be subject to azo reductive cleavage resulting in the release of aromatic amines. In particular, azo reductive cleavage of Solvent Red 23 may result in the release of 4-aminoazobenzene, a substance that has been classified by the International Agency for Research on Cancer and the European Commission on the basis of its carcinogenicity. Solvent Red 23 is also structurally similar to another azo dye that has been demonstrated to induce liver neoplasias in orally exposed male and female rats in a dose dependent manner and classified as a mutagen and carcinogen by the European Commission. Based on consideration of exposure potential to the general population from use of cosmetics and personal care products containing Solvent Red 23, and evidence of potential genotoxicity and carcinogenicity for which there may be a probability of harm at any level of exposure, Solvent Red 23 is concluded to be entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

Where relevant, research and monitoring will support verification of assumptions used during the screening assessment and, where appropriate, the performance of potential control measures identified during the risk management phase.

Based on the information available, it is concluded that 2-naphthalenol, 1-[[4-(phenylazo)phenyl]azo]- meets one or more of the criteria set out in section 64 of CEPA 1999.

Introduction

The *Canadian Environmental Protection Act, 1999* (CEPA 1999) (Canada 1999) requires the Minister of the Environment and the Minister of Health to conduct screening assessments of substances that have met the categorization criteria set out in the Act to determine whether these substances present or may present a risk to the environment or to human health.

Based on the information obtained through the categorization process, the Ministers identified a number of substances as high priorities for action. These include substances that

- met all of the ecological categorization criteria, including persistence (P), bioaccumulation potential (B) and inherent toxicity to aquatic organisms (iT), and were believed to be in commerce in Canada; and/or
- met the categorization criteria for greatest potential for exposure (GPE) or presented an intermediate potential for exposure (IPE), and had been identified as posing a high hazard to human health based on classifications by other national or international agencies for carcinogenicity, genotoxicity, developmental toxicity or reproductive toxicity.

The Ministers therefore published a notice of intent in the *Canada Gazette*, Part I, on December 9, 2006 (Canada 2006a), that challenged industry and other interested stakeholders to submit, within specified timelines, specific information that may be used to inform risk assessment, and to develop and benchmark best practices for the risk management and product stewardship of those substances identified as high priorities.

The substance , 2-Naphthalenol, 1-[[4-(phenylazo)phenyl]azo]-, (which will be referred to as Solvent Red 23 for the purposes of this document) had been identified as a high priority for assessment of ecological risk as it had been found to be persistent, bioaccumulative and inherently toxic to aquatic organisms, and is believed to be in commerce in Canada. The Challenge for this substance was published in the *Canada Gazette* on May 31, 2008 (Canada 2008a, 2008b). A substance profile was released at the same time. The substance profile presented the technical information available prior to December 2005 that formed the basis for categorization of this substance. As a result of the Challenge, submission of information pertaining to the uses of Solvent Red 23 and some of its formulation products were received.

Although Solvent Red 23 was determined to be a high priority for assessment with respect to the environment, it did not meet the criteria for GPE or IPE and high hazard to human health based on classifications of other national or international agencies for carcinogenicity, genotoxicity, developmental toxicity or reproductive toxicity.

Screening assessments focus on information critical to determining whether a substance meets the criteria as set out in section 64 of CEPA 1999. Screening assessments examine

scientific information and develop conclusions by incorporating a weight-of-evidence approach and precaution[1].

This final screening assessment includes consideration of information on chemical properties, hazards, uses and exposure, including any information submitted under the Challenge. Data relevant to the screening assessment of this substance were identified in original literature, review and assessment documents, stakeholder research reports and from recent literature searches, up to July 2010. Key studies were critically evaluated; modelling results have also been used to reach conclusions. When available and relevant, information presented in hazard assessments from other jurisdictions was considered. This final screening assessment does not represent an exhaustive or critical review of all available data. Rather, it presents the most critical studies and lines of evidence pertinent to the conclusion.

This final screening assessment was prepared by staff in the Existing Substances Programs at Health Canada and Environment Canada and incorporates input from other programs within these departments. The ecological portion of this assessment has undergone external written peer review/consultation. Comments on the technical portions relevant to human health were received from scientific experts selected and directed by Toxicology Excellence for Risk Assessment (*TERA*) and included comments by Dr. Larry Claxton, Dr. Bernard Gadagbui, Dr. Pertti Hakkinen, Dr. Glenn Talaska, and Dr. Pam Williams.

Additionally, the draft of this screening assessment was subject to a 60-day public comment period. While external comments were taken into consideration, the final content and outcome of the screening assessment remain the responsibility of Health Canada and Environment Canada. Approaches used in the screening assessments under the Challenge have been reviewed by an independent Challenge Advisory Panel.

The critical information and considerations upon which this assessment is based are summarized below.

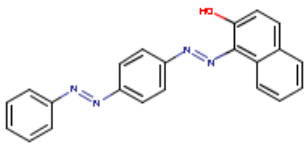
[1] A determination of whether one or more of the criteria of section 64 are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposures in the general environment. For humans, this includes, but is not limited to, exposures from ambient and indoor air, drinking water, foodstuffs, and the use of consumer products. A conclusion under CEPA 1999 on the substances in the Chemicals Management Plan (CMP) Challenge Batches 1-12 is not relevant to, nor does it preclude, an assessment against the hazard criteria specified in the Controlled Products Regulations, which is part of the regulatory framework for the Workplace Hazardous Materials Information System [WHMIS] for products intended for workplace use. Similarly, a conclusion based on the criteria contained in section 64 of CEPA 1999 does not preclude actions being taken under other sections of CEPA or other Acts.

Substance Identity

Substance Name

For the purposes of this document, 2-Naphthalenol, 1-[[4-(phenylazo)phenyl]azo]- will be referred to as Solvent Red 23, its Colour Index name (Colour Index Constitution Number: 26100; CII 2002). Information on this substance's identity is shown in Table 1 below.

Table 1. Substance identity for Solvent Red 23

Chemical Abstracts Service Registry Number (CAS RN)	**85-86-9**
DSL name	**2-Naphthalenol, 1-[[4-(phenylazo)phenyl]azo]-**
Inventory names[1]	*2-Naphthalenol, 1-[[4-(phenylazo)phenyl]azo]-* (TSCA, AICS, PICCS, ASIA-PAC, NZIoC) *1-(4-(phenylazo)phenylazo)-2-naphthol* (EINECS) *Solvent Red 23* (ENCS, PICCS) *C.I. solvent red 023* (ECL) *Sudan III* (PICCS) *naphth-2-ol, 1-((4-phenylazo)phenyl)azo)-* (PICCS)
Other names	*1-(p-Phenylazophenylazo)-2-naphthol; 111440 Red; 2-Naphthol, 1-(p-phenylazophenylazo)-; Brasilazina Oil Scarlet; C.I. 26100; C.I. Solvent Red 23; Certiqual Oil Red; D and C Red No. 17; Fast Oil Scarlet III; Fat Red Bluish; Fat Red HRR; Fat Red R; Fat Red RS; Fat Scarlet LB; Fat Soluble Red Zh; FD And C Red No. 17; Grasal Brilliant Red G; Grasan Brilliant Red G; Japan Red 225; Japan Red No. 225; NSC 65825; NSC 8995; Oil Red 3G; Oil Red AS; Oil Red Extra; Oil Scarlet G; Organol Red BS; Organol Scarlet; Red No. 225; Red Zh; Silotras Scarlet TB; Somalia Red III; Stearix Scarlet; Sudan 3; Sudan P III; Sudan Red III; Tetrazobenzene-b-naphthol; Toney Red*
Chemical group	Azo compounds
Chemical sub-group	Disazo compounds
Chemical formula	$C_{22}H_{16}N_4O$
Chemical structure	
SMILES[2]	Oc(ccc(c1ccc2)c2)c1N=Nc(ccc(N=Nc(cccc3)c3)c4)c4
Molecular mass	352.40 g/mol

[1] National Chemical Inventories (NCI). 2006: AICS (Australian Inventory of Chemical Substances); ASIA-PAC (Asia-Pacific Substances Lists); ECL (Korean Existing Chemicals List); EINECS (European Inventory of Existing Commercial Chemical Substances); ENCS (Japanese Existing and New Chemical Substances); NZIoC (New Zealand Inventory of Chemicals); PICCS (Philippine Inventory of Chemicals and Chemical Substances); and TSCA (U.S. Toxic Substances Control Act Chemical Substance Inventory).

[2] Simplified Molecular Line Input Entry System

Physical and Chemical Properties

Solvent Red 23 is a disazo dye. The two azo bonds (–N=N–) of this molecule are functional groups that produce colour (Danish EPA 1999). In addition to chemical structure, dyes may be classified according to their industrial applications and the methods by which they are applied to the substrate of interest (ETAD 1995). This classification system tends to reflect groupings based on physical and chemical behaviour. A brief discussion of the uses of this dye can be found later in this document under the Uses section.

Few experimental data on the physical and chemical properties of Solvent Red 23 are available. At the Environment Canada-sponsored Quantitative Structure-Activity Relationship (QSAR) Workshop in 1999, invited modelling experts identified many structural classes of pigments and dyes as “difficult to model” using QSARs (Environment Canada 2000). The physical and chemical properties of many of the structural classes of pigments and dyes are not amenable to model prediction because they are considered “out of the model domain of applicability” (e.g., structural and/or property parameter domains). Therefore, to determine potential utility, the domains of applicability of QSAR models to pigments and dyes are reviewed on a case-by-case basis.

For this assessment it is considered that certain QSAR models used to predict physical and chemical properties that lack comparable substances to Solvent Red 23 in their domain of applicability, may produce results with a high degree of uncertainty. Consequently, a "read-across" approach has been used to determine the approximate physical and chemical properties in Table 2. These properties were subsequently considered in evaluating various lines of evidence in this assessment. Table 2 shows some experimental and extrapolated physical and chemical properties of Solvent Red 23 and analogues.

An analogue is a chemical which is structurally similar to the substance under assessment and is therefore expected to have similar physical-chemical properties, behaviour in the environment and/or toxicity. Where there are experimental data for a given parameter for an analogue substance, these can be used directly or with adjustment as an estimate of that parameter value for the substance under assessment.

To find acceptable analogues, a review of data for several disperse azo dyes was performed (Anliker et al. 1981; Anliker and Moser 1987; Baughman and Perenich 1988; ETAD 1995; Brown 1992; Yen et al. 1989; Sijm et al. 1999). These compounds have structural similarities to Solvent Red 23 but also share other important attributes that contribute to their suitability as analogues. This includes properties affecting their fate in the environment such as high molecular weights, generally >300 g/mol, high cross-sectional diameters (1.31-2.19 nm), solid particulate structures, decomposition at greater than 120 deg C, and "dispersibility" in water (i.e., not truly "soluble"). In addition, they have a negligible vapour pressure and are stable under environmental conditions as they are designed to be so. Additional analogues have been chosen for the human health assessment, where data exist (see Potential to Cause Harm to Human Health section for further rationale and discussion).

Since some of the studies for the disazo dyes were conducted under non-relevant environmental conditions (e.g., high temperature) and/or had limited information on which to assess their reliability, data for monoazo disperse dyes and azo disperse dyes in general, are also presented in Table 2.

Table 2. Experimental physical and chemical properties for Solvent Red 23 and relevant analogues.

Property	Type[1]	Value	Temperature (°C)	Reference
Physical state	Analogue Disperse Orange 29	Powder		Study Submission 2008a
Decomposition point[2] (ºC)	Solvent Red 23	195		PhysProp 2006
	Analogue Sudan IV (also known as Solvent Red 24)	185		MITI 1992
	Analogue Disperse Orange 29	223		ETAD 2005
	Analogue Disperse Yellow 23	158 178		Odabaşoğlu et al. 2003; Datyner 1978

	Analogue Disperse Orange 13	153 to 156.5		Nishida et al. 1989
	Analogue Disperse Orange 30	126.9 to 128.5		ETAD 2005
	Analogue Disperse Blue 79	157		PhysProp 2006
	Analogue Disperse Blue 79:1	132 153		Sijm et al. 1999; Yen et al. 1989
	Read-across for azo disperse dyes	117 to 175 74 to 236		Anliker and Moser 1987; Baughman and Perenich 1988
Boiling point[3] (ºC)	Not applicable			
Density (kg/m^3)	Not available			
Vapour pressure (Pa)	Analogue Disperse Orange 13	0.18 to 0.42[4]	191.5 to 211	Nishida et al. 1989
	Analogue Disperse Blue 79	4.53×10^{-7}		Clariant 1996
	Read-across for azo disperse dyes	5.3×10^{-12} to 5.3×10^{-5}) (4×10^{-14} to 4×10^{-7} mm Hg)	25	Baughman and Perenich 1988
Henry's Law constant ($Pa \cdot m^3/mol$)	Read-across for azo disperse dyes	10^{-8} to 10^{-1} (10^{-13} to 10^{-6} $atm \cdot m^3/mol$)[5]		Baughman and Perenich 1988

Log K_{ow} (Octanol-water partition coefficient) (dimensionless)	Analogue Disperse Orange 29	4.6[6]		Study Submission 2008a
	Analogue Disperse Blue 79	4.1; 4.3[7]		Clariant 1996; Brown 1992
	Analogue Disperse Blue 79:1	4.4; 4.8		Sijm et al. 1999; Yen et al. 1989
	Analogue Disperse Orange 30	4.2[8]		Brown 1992
	Read-across for azo disperse dyes	1.79 to 5.1		Baughman and Perenich 1988
		>2 to 5.1		Anliker et al. 1981; Anliker and Moser 1987
Log K_{oc} (Organic carbon-water partition coefficient) (dimensionless)	Read-across, calculated	3.4 to 4.2[9]		Baughman and Perenich 1988
Water solubility (mg/L)	Analogue Disperse Orange 13	0.345		PhysProp 2006
	Analogue Disperse Orange 29	42.9[6]		Study Submission 2008a
		0.0037	25	Baughman et al. 1996 (estimated)
	Analogue Disperse Yellow 23	0.00006	25	Baughman and Perenich 1988
		0.00052		Baughman et al. 1996 (estimated)

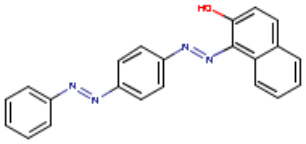
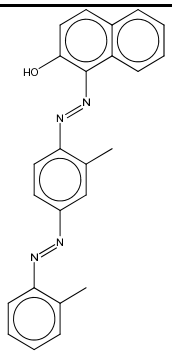
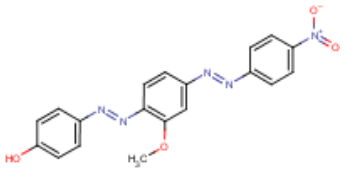
		15.7 to 34.8[4]	130	Braun 1991
	Analogue Disperse Yellow 68	16.6[4]	125	Prikryl et al. 1979
	Analogue Disperse Blue 79	0.0054	25	Clariant 1996
		0.02[7]		Brown 1992
	Analogue Disperse Blue 79:1	0.02		Sijm et al. 1999
		0.0052		Yen et al. 1989
		0.00063[4]	100 to 125	Baughman and Perenich 1988
	Disperse Orange 30	0.07[8]		Brown 1992
	Read-across for azo disperse dyes	<0.01	20	Anliker and Moser 1987
		Substantially water insoluble		ETAD 1995
		1.2×10^{-5} to 35.5 (4×10^{-11} to 1.8×10^{-4} mol/L)		Baughman and Perenich 1988
n-octanol solubility (mg/L)	Analogue Disperse Orange 29	5086		ETAD 2005
	Analogue Disperse Orange 30	576		ETAD 2005
	Analogue Disperse Blue 79:1	14		Sijm et al. 1999

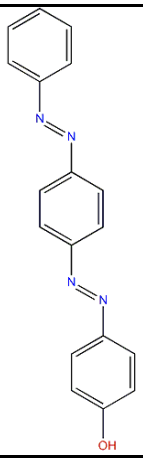
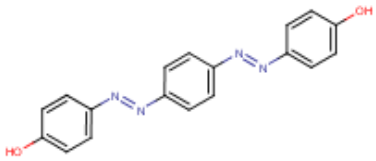
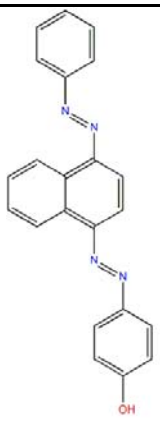
	Read-across for azo disperse dyes	81 to 2100	20	Anliker and Moser 1987
pK_a (Acid dissociation constant) (dimensionless)	Solvent Red 23	13.5		ACD/pK_a DB 2005
	Disperse Yellow 23	8.1		Haag and Mill 1987

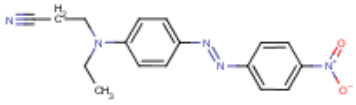
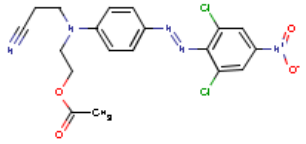
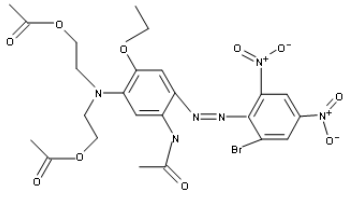
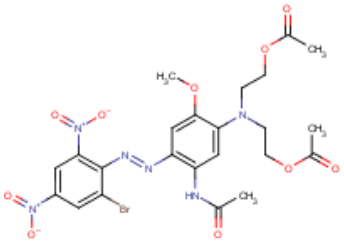
[1] Analogues of Solvent Red 23 are indicated in Table 2 CAS RN, molecular structures, molecular weight and cross-sectional diameter of analogues are provided in Tables 3a and 3b.
[2] The phrase "melting point" is used, but this may be better referred to as a decomposition point because disperse dyes are known to char at high temperatures (greater than 200°C) rather than melt.
[3] Boiling point is generally not applicable for disperse dyes. For powder dyes, charring or decomposition occurs at high temperatures instead of boiling. For liquids and pastes, boiling will only occur for the solvent component, while the unevaporated solid will decompose or char (ETAD 1995).
[4] Note that the water solubility tests in these studies were performed at very high temperatures and so are higher than expected at room temperature.
[5] Solubility values of five azo disperse dyes (Disperse Orange 3, Disperse Red 1, Solvent Yellow 2, Dis. A. 5, Dis. A. 7) at 25 and 80°C were used by Baughman and Perenich (1988) to calculate Henry's Law constants for these dyes. These values are presented here as a range to illustrate the expected Henry's Law constant for disazo dyes.
[6] The study indicates that the Disperse Orange 29 used in the test was a dispersion of 20% dye stuff that was tested (70% water and 10% Reax).
[7] The study indicates that the Disperse Blue 79 used in the test had a purity (as organic materials) of 76% and a dispersion of 20% dye stuff.
[8] The study indicates that the Disperse Orange 30 used in the test had a purity (as organic materials) of 73% and a dispersion of 20% dye stuff.
[9] Log K_{oc} values are based on calculations by Baughman and Perenich (1988) using a range of measured solubility for commercial dyes and an assumed melting point of 200°C.

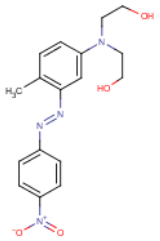
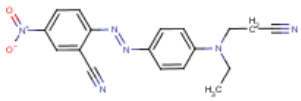
Because of the paucity of empirical data for Solvent Red 23 and the error associated with model predictions for disperse dyes, selected empirical physical and chemical properties (Table 2), bioaccumulation data (Table 5a and 5b) and aquatic toxicity data (Table 6) were used to support the weight of evidence and conclusions in this screening assessment. Specifically, data were obtained for a similar solvent dye (Sudan Red IV/Solvent Red 24), 4 structurally similar disazo disperse dyes (Disperse Orange 29, Disperse Yellow 23, Disperse Yellow 68 and Disperse Orange 13) and 6 structurally similar monoazo dyes (Disperse Blue 79, Disperse Blue 79:1, Disperse Orange 30, Disperse Red 73, Disperse Orange 25 and Disperse Red 17). Substance identity information, as well as empirical data for analogues used in this report, are presented in Table 3a while the molecular weights and cross-sectional diameters are presented in Table 3b.

Table 3a. Structural analogues for Solvent Red 23 considered for ecological assessment[1]

Common name (CAS RN)	DSL name	Structure	Major structural similarities and differences with Solvent Red 23	Available empirical data[1]
Solvent Red 23 (85-86-9)	2-Naphthalenol, 1-[[4-(phenylazo)phenyl]azo]-		Not applicable (same substance).	Melting Point
Sudan IV (also known as Solvent Red 24) (85-83-6)	2-Naphthalenol, 1-[[2-methyl-4-[(2-methylphenyl)azo]phenyl]azo]-		Similarity: Aromatic disazo compound with a naphthalene ring and a hydroxyl group attached. Same numbers of rings. Differences: Two additional methyl groups – one each attached to the single rings.	Melting Point, toxicity, BCF.
Disperse Orange 29 (19800-42-1)	Phenol, 4-[[2-methoxy-4-[(4-nitrophenyl)azo]phenyl]azo]-		Similarity: Aromatic disazo compound with a hydroxyl group Differences: Solvent Red 23 contains a naphthalene ring and no terminal nitro group or ether group.	Physical state, melting point, log K_{ow}, water solubility, n-octanol solubility

Common name (CAS RN)	DSL name	Structure	Major structural similarities and differences with Solvent Red 23	Available empirical data[1]
Disperse Yellow 23 (6250-23-3)	Phenol, 4-[[4-(phenylazo)phenyl] azo]-		Similarity: Aromatic disazo compound with a hydroxyl group Differences: Disperse Yellow 23 does not contain a naphthalene ring.	Melting point, water solubility, pKa and aquatic toxicity
Disperse Yellow 68 (21811-64-3)	Phenol, 4,4'-[1,4-phenylenebis(azo)] bis		Similarity: Aromatic disazo compound with a hydroxyl group Differences: Disperse Yellow 68 does not contain a naphthalene ring and has an additional hydroxyl group.	Water solubility
Disperse Orange 13 (6253-10-7)	Phenol, 4-[[4-(phenylazo)-1-naphthalenyl]azo]-		Similarity: Aromatic disazo compound with a naphthalene ring and a hydroxyl group Differences: Disperse Orange 13 has a naphthalene ring in a different position	Melting point, water solubility, vapour pressure

Common name (CAS RN)	DSL name	Structure	Major structural similarities and differences with Solvent Red 23	Available empirical data[1]
Disperse Orange 25 (31482-56-1)	3-(Ethyl(4-((4-nitrophenyl)azo)phenyl)amino)propanenitrile		Similarity: Aromatic azo compound. Differences: No second azo group, Disperse Orange 25 contains a nitrile, nitro and amine group and does not have a naphthalene ring.	Aquatic toxicity
Disperse Orange 30 (5261-31-4)	Propanenitrile, 3-[[2-(acetyloxy)ethyl][4-[(2,6-dichloro-4-nitrophenyl)azo]phenyl]amino]-		Similarity: Aromatic azo compound. Differences: No second azo group, Disperse Orange 30 contains nitrile, carboxylic, nitro and amine functional groups as well as two chlorines.	Bioaccumulation, aquatic toxicity, log K_{ow}
Disperse Blue 79 (12239-34-8)	Acetamide, *N*-[5-[bis[2-(acetyloxy)ethyl]amino]-2-[(2-bromo-4,6-dinitrophenyl)azo]-4-ethoxyphenyl]-		Similarity: Aromatic azo compound. Differences: No second azo group, Disperse Blue 79 contains two carboxylic groups, two nitro functional groups, an aniline with two carbon chains, an amine functional group and a bromine moiety.	Melting point, vapour pressure, log K_{ow}, water solubility, aquatic toxicity
Disperse Blue 79:1 (3618-72-2)	Acetamide, N-[5-[bis[2-(acetyloxy)ethyl]amino]-2-[(2-bromo-4,6-dinitrophenyl)azo]-4-methoxyphenyl]-		Similarity: Aromatic azo compound. Differences: No second azo group, Disperse Blue 79:1 contains two carboxylic groups, two nitro functional groups, an amine functional group and an aniline with two short carbon chains.	Melting point, log K_{ow}, water solubility, bioaccumulation, aquatic toxicity

Common name (CAS RN)	DSL name	Structure	Major structural similarities and differences with Solvent Red 23	Available empirical data[1]
Disperse Red 17 (3179-89-3)	Ethanol, 2,2'-((3-methyl-4-(2-(4-nitrophenyl) diazenyl)phenyl) imino)bis-		Similarity: Aromatic azo compound with a hydroxyl group. Differences: No second azo group, Disperse Red 17 contains an additional hydroxyl group and aniline with two short carbon chains	Aquatic toxicity
Disperse Red 73 (16889-10-4)	2-((4-((2-Cyanoethyl) ethylamino)phenyl) azo)-5-nitro benzonitrile		Similarity: Aromatic azo compound. Differences: No second azo group, Disperse Red 73 contains two nitrile functional groups, nitro and aniline group as well as two short carbon chains.	Aquatic toxicity

[1] Note that analogues used for mammalian toxicity data are provided in Table 7 and Appendix 5.

Table 3b. Comparison of the molecular mass and cross-sectional diameter of the monoazo and disazo disperse dye structural analogues

	CAS RN	Common name	Molecular mass (g/mol)	Minimum–maximum D_{max} (nm)[1]
Disazo solvent and disperse dyes	85-86-9	Solvent Red 23	352	1.50–2.03
	85-83-6	Sudan IV	380	1.50-2.05
	19800-42-1	Disperse Orange 29	377	1.56–2.19
	6250-23-3	Disperse Yellow 23	302	1.50–2.07
	6253-10-7	Disperse Orange 13	352	1.56–2.07
	21811-64-3	Disperse Yellow 68	318	2.09–2.14
Monoazo dye analogues	12239-34-8	Disperse Blue 79	639	1.69–2.05
	3618-72-2	Disperse Blue 79:1	625	1.43–2.03
	5261-31-4	Disperse Orange 30	450	1.75–1.98
	16889-10-4	Disperse Red 73	348	1.31–1.93
	31482-56-1	Disperse Orange 25	323	1.37–1.95
	3179-89-3	Disperse Red 17	344	1.41–1.86

[1] Based on range of maximum diameters (D_{max}) for conformers calculated using CPOPs (2008)

It should be noted that there are several uncertainties associated with the use of physical and chemical, toxicological, and bioaccumulation data available for the substances. All

these substances share the same chemical class – azo compounds (one subset has two azo bonds and another has one azo bond) – and are used for similar industrial purposes (i.e., disperse dyes and a solvent dye). However, there are differences between these substances associated with their unique functional groups (see Table 3a) and some of their molecular sizes. In spite of the fact that some of these monoazo dyes have larger molecular weights than the disazo dyes, their comparable physical state, melting points, water solubility, log K_{ow} values and cross-sectional diameters (Tables 3b) provide a reasonable basis to conclude that the monoazo dyes will behave similarly to the disazo dyes in the environment and present an approximately equal bioavailability, and that their use as analogues for Solvent Red 23 is therefore acceptable.

Sources

Solvent Red 23 is not naturally produced in the environment.

Recent information was collected through industry surveys conducted for the years 2005 and 2006 under *Canada Gazette* notices issued pursuant to section 71 of CEPA 1999 (Canada 2006b and 2008b). These notices required submission of data on the Canadian manufacture, import and use of Solvent Red 23. In the notice for 2006, data were also required on use quantities of this dye. In association with the section 71 notices for 2005 and 2006, companies that did not meet the mandatory reporting requirements but had a business interest in Solvent Red 23 were invited to identify themselves as stakeholders.

In the Declaration of Stakeholder-Interest form associated with the section 71 survey for 2006, nine companies reported a stakeholder interest in Solvent Red 23 despite not meeting mandatory reporting requirements (Environment Canada 2008a). In more than one case, the interest involved import or current use of the substance in Canada below the threshold. In 2005, four companies each reported importing 100–1000 kg of Solvent Red 23 (Environment Canada 2006). Eight companies identified themselves as having a stakeholder interest in Solvent Red 23 in 2005 (Environment Canada 2006).

During the development of the Domestic Substances List (DSL) the quantity of Solvent Red 23 reported as being manufactured, imported or in commerce in 1986 was 2 200 kg (Environment Canada 1988).

Solvent Red 23 has been identified as a low production volume (LPV) chemical by the European Union (EU), indicating that production within the EU is estimated to be between 10 and 1000 tonnes per year (ESIS 2008). Solvent Red 23 was also used in Sweden from 1999 to 2006 and in Denmark from 2003 to 2006 (SPIN 2008).

Products containing Solvent Red 23 may enter Canada even if they are not identified as such in the section 71 survey because they may be imported unknowingly in manufactured items, or in quantities below the 100 kg reporting threshold for the survey.

Uses

Information on uses for the 2005 and 2006 calendar years was gathered in response to the CEPA 1999 section 71 notices (Canada 2006b, 2008b). In 2005, companies importing Solvent Red 23 indicated that they were engaged in retailing cosmetics, perfumes, toiletries and personal grooming products or providing hair care or aesthetic services (Environment Canada 2006). In 2006, none of the companies that reported importing or using Solvent Red 23 through the Declaration of Stakeholder Interest indicated the type of use (Environment Canada 2008a). During the DSL nomination (1984–1986), the DSL use codes for "Colourant – pigment/stain/dye/ink", "fragrance/perfume/deodorizer/flavouring agent", "cosmetics", "health and veterinary", "pharmaceuticals", "pigment, dye and printing ink" and "textile, product" were identified for Solvent Red 23.

Solvent Red 23 is currently used in cosmetic products in several jurisdictions including Canada, the United States and in Europe. In Canada, Solvent Red 23 is found in Health Canada's cosmetics notification system (CNS) database in approximately 300 cosmetics and personal care products as "Solvent Red 23", "D&C Red no. 17" or "CI 26100" (CNS 2009).

Solvent Red 23 is listed on the Food and Drugs Regulations under section C.01.040.2(4)(a) as a colouring agent permitted in drugs for external use under the name Toney Red (D&C Red No. 17; C.I. No. 26100) (Canada 1978). Solvent Red 23 is listed in the Therapeutic Products Directorate's internal Non-Medicinal Ingredients Database as a colourant present in one liquid disinfectant used in hospitals and in premises in which food is manufactured, prepared or kept, but has not been identified to be present in pharmaceutical or veterinary drugs (2008 email from Therapeutic Products Directorate, Health Canada, to Risk Management Bureau; unreferenced). Solvent Red 23 is also listed in the Natural Health Products Ingredients Database (NHPID) as a permitted non-medicinal ingredient to be used as a colouring agent for external use only (NHPID 2010). Solvent Red 23 is listed in the Licensed Natural Health Products Database (LNHPD) and therefore is present in licensed natural health products (LNHPD 2010). Although the presence of Solvent Red 23 in spices has previously been reported, this substance is not a permitted food colourant in the European Union, the United States or Canada (European Community 1994; US FDA 2007; Health Canada 2007). Solvent Red 23 is not listed as a permitted food additive under the *Food and Drug Regulations* nor has it been identified for use in food packaging applications (Health Canada 2007).

There are current uses of Solvent Red 23 in cosmetics and drugs in other jurisdictions. Solvent Red 23 is currently approved in the United States as D&C Red No. 17 for use as a colourant in externally applied drugs and cosmetics and as a colourant in contact lenses (US FDA 2009). In Europe, Solvent Red 23 (as CI 26100) is also approved for use as a colourant in cosmetics that are not intended to come into contact with mucous membranes (Cosmetics Directive Annex IV). While Solvent Red 23 has been reported to be found in hair dyes, this use is currently prohibited in Europe (Cosmetics Directive Annex II) (European Commission 2010).

Solvent Red 23 is listed in Canada as a formulant in insecticide/herbicide products (2009 email from PMRA to Existing Substances Risk Assessment Bureau, Health Canada; unreferenced) and is also on the list of inert ingredients permitted for use in pesticide products applied to non-food use sites, such as ornamental plants and highway rights-of-way in the United States (US EPA 2008).

Additional possible uses of Solvent Red 23 include biological stains (O'Neil 2006; ProSciTech 2006), oils, fats, waxes and plastics (CII 2002-).

In addition, the European Commission REACH (Registration, Evaluation, Authorisation and Restriction of Chemical substances) regulation (European Commission 2006), specifically Point 43 of Annex XVII, restricts certain "Azocolourants and Azodyes" in certain textiles that may release one of 22 aromatic amines by cleavage of the azo bond. While Solvent Red 23 would theoretically be reductively cleaved to one of the 22 restricted aromatic amines (i.e., 4-aminoazobenzene CAS RN 60-09-3), based on the available information, the current global use pattern of this substance does not at this time indicate its presence in textiles and leather products.

Releases to the Environment

There was insufficient information on which to base a life cycle analysis for Solvent Red 23. However, based on its use pattern in personal care products, it is anticipated that 100% of products containing Solvent Red 23 could be released to sewer, surface water or land during their use. Once released to surface or sewer water, due to its physical and chemical properties Solvent Red 23 is expected to bind to sediment in aquatic environments and to sludge in wastewater treatment systems.

Based on the above, sewer water is the medium potentially receiving the greatest proportion of Solvent Red 23 during product use. It is anticipated that the majority of the substance, bound to sewage sludge from down-the-drain releases of personal care products to sewage treatment plants (STPs), will be sent entrained in sludge to landfills. In addition to being landfilled, some of the biosolids from wastewater treatment facilities may be applied to land as a fertilizer or soil conditioner for uses in agriculture, forestry and reclamation and a small percentage may be incinerated.

Environmental Fate

Solvent Red 23 is expected to be released to wastewater effluents during industrial processing and down-the-drain uses. The high read-across log K_{ow} value (4.6) and log K_{oc} (3.4 to 4.2) values (see Table 2) indicate that this substance may have affinity for solids. However, the log K_{oc} values are calculated not strictly experimental (see footnote 3 below Table 2) and the adsorption potential of disperse particulate dye structures is

generally not well understood; therefore the degree to which this particular behaviour applies is uncertain.

According to aerobic biodegradation models, Solvent Red 23 is not expected to biodegrade quickly (see Table 4 below). This substance may inadvertently be applied to land as a fertilizer or soil conditioner for uses in agriculture, forestry and reclamation as a component of biosolids. Solvent Red 23 may be deposited directly to land through its use as a pesticide formulant.

Given an experimental pKa of 8.1 for the analogue Disperse Yellow 23 and an estimated pKa value of 13.5 for Solvent Red 23 (Table 2), this chemical is expected to behave as a weak acid and be partially ionized in water at the higher end of environmentally relevant pHs (8–9). However, given the expected low water solubility of Solvent Red 23 (Table 2) and its particulate state, it is unlikely that ionization at elevated pH will have significant impact on the partitioning or water solubility of this substance. Instead, when released into water, this substance is expected to be mostly present as a particulate solid or adsorbed to suspended particles and eventually sink to surface bed sediments where it is expected to remain in a relatively biologically unavailable form. It has been stated generally that, due to the recalcitrant nature of azo dyes in aerobic environments, they eventually end up in anaerobic sediments, shallow aquifers and in groundwater (Razo-Flores et al. 1997). However, since Solvent Red 23 has low water solubility and a relatively high K_{oc} it is not likely to leach from sediments and soils.

The rate of volatilization from the surface of water is proportional to the Henry's law constant (Baughman and Perenich 1988). Baughman and Perenich (1988) also state that volatilization from aquatic systems will not be an important loss process for disperse dyes, which agrees with the low to negligible read-across Henry's Law constant value (10^{-8} to 10^{-1} Pa•m^3/mol; Table 2). Transport in air due to the loss of this substance from moist and dry soil surfaces is not likely to be important for this substance as indicated by very low read-across vapour pressures (5.33×10^{-12} to 5.33×10^{-5} Pa; Table 2). These data are consistent with the physical state (solid particle) of the disazo dyes which makes them unlikely candidates for volatilization. The experimental vapour pressure for Disperse Orange 13 is not a useful indicator of volatilization for Solvent Red 23 since it was measured at an elevated temperature.

Persistence and Bioaccumulation Potential

Environmental Persistence

Dyes must have a high degree of chemical and photolytic stability in order to be useful, so most are generally considered non-degradable under environmentally relevant aerobic conditions (Danish EPA 1999; ETAD 1995). Studies applying commonly accepted screening tests (e.g., OECD guidelines) for ready and inherent biodegradability have confirmed this point (ETAD 1992; Pagga and Brown 1986). Abiotic degradation, including photolysis and hydrolysis, is not thought to play a significant role in the environmental fate of azo dyes (Danish EPA 1999), although one study showed strongly accelerated photo decomposition of azo dyes in the presence of natural humic materials (Brown and Anliker 1988).

Biotic degradation of azo dyes may take place relatively rapidly under anaerobic or reducing conditions (Baughman and Weber 1994; Danish EPA 1999; ETAD 1995; Isik and Sponza 2004; Yen et al. 1991). Permeability of the bacterial cell wall has been found to be the rate-limiting step in the reduction process (Danish EPA 1999). Azo dyes have a high tendency to cleave at the azo bond with the formation of aromatic amines (Danish EPA 1999; Hunger 2005). The carcinogenic potential of aromatic amines varies considerably with molecular structure, with carcinogenic breakdown products being associated with the moieties of benzidine, aniline, toluene or naphthalene. However, the formation of such metabolites in deep anoxic sediments would typically not result in exposure to aquatic organisms. Total mineralization or further degradation of these metabolites could take place if they are transferred (e.g., by sediment resuspension) to aerobic environments (Danish EPA 1999; Isik and Sponza 2004). Aromatic amines may also be present as impurities in commercially available azo dyes, although the metabolic cleavage of azo dyes is the main source of these compounds (Danish EPA 1999).

A bioelimination study in water was submitted for the analogue Disperse Yellow 23 indicating that it undergoes 51% degradation in 14 days (Study Submission 2008b). However, due to lack of experimental details, this study was considered to have low reliability and its experimental result could not be used to support the persistence assessment of Solvent Red 23 (see Robust Study Summary in Appendix 1). Other than this study, no experimental or read-across degradation data for Solvent Red 23 or analogues have been identified. No environmental monitoring data relating to the persistence of these dyes in the Canadian environment (air, water, soil, sediment) have been identified.

Given the expected release of Solvent Red 23 as a dye into wastewater, persistence was primarily examined using predictive QSAR models for aerobic biodegradation in water. These models are considered acceptable for use in this situation as they are based on chemical structure and the disazo structure is represented in the training sets of all the BIOWIN models used, thereby increasing the reliability of the predictions (Environment

Canada 2007). The following analysis applies primarily to the portion of this substance that is present in the environment in the dissolved form, recognizing that a significant proportion would also likely exist in dispersed form as solid particles. Solvent Red 23 and its analogues do not contain functional groups expected to undergo hydrolysis in aerobic environments (dyes are designed to be stable in aqueous conditions). Table 4 summarizes the results of available QSAR models for aerobic biodegradation in water.

Table 4. Modelled data for degradation of Solvent Red 23

Fate Process	Model and model basis	Model Result and Prediction	Extrapolated Half-life (days)
Primary biodegradation			
Biodegradation (aerobic)	BIOWIN 2008[1] Sub-model 4: Expert Survey (qualitative results)	3.3[2] "biodegrades slowly"	≥182
Ultimate biodegradation			
Biodegradation (aerobic)	BIOWIN 2008[1] Sub-model 3: Expert Survey (qualitative results)	1.90[2] "biodegrades very slowly"	≥182
Biodegradation (aerobic)	BIOWIN 2008[1] Sub-model 5: MITI linear probability	-0.24[3] "biodegrades very slowly"	≥182
Biodegradation (aerobic)	BIOWIN 2008[1] Sub-model 6: MITI non-linear probability	0[3] "biodegrades slowly"	≥182
Biodegradation (aerobic)	TOPKAT 2004 Probability	n/a[4] "biodegrades slowly	≥182
Biodegradation (aerobic)	CATABOL 2004-2008 % BOD[5] (biological oxygen demand)	% BOD = 0 "biodegrades very slowly"	≥182

[1] EPIsuite (2008)
[2] Output is a numerical score from 0 to 5.
[3] Output is a probability score.
[4] n/a: not available (out of model domain)
[5] BOD: Biological oxygen demand

The results from Table 4 reveal that all biodegradation models (BIOWIN 3, 5, 6 and CATABOL) suggest that Solvent Red 23 biodegrades slowly aerobically in water. In fact both BIOWIN 5 and 6 probability results are much less than 0.3, the cut-off suggested by Aronson et al. (2006) to identify substances as having a half-life ≥60 days (based on the MITI probability models). Furthermore, both other ultimate degradation models, BIOWIN 3 and CATABOL, predict that Solvent Red 23 will be persistent in water. TOPKAT (2004) also predicts slow biodegradation but the model output is unreliable as the structure is outside of the domain of model applicability.

When the results of the probability and the other ultimate degradation models are considered, there is model consensus suggesting that the ultimate biodegradation half-life in water is ≥182 days. This finding is consistent with what would be expected for these chemical structures (i.e., few degradable functional groups, solid sparingly soluble particle).

Using an extrapolation ratio of 1:1:4 for a water:soil:sediment biodegradation half-life (Boethling et al. 1995), the ultimate degradation half-life in aerobic soil is ≥182 days and the half-life in aerobic sediments is ≥365 days. This suggests that Solvent Red 23 is expected to be persistent in soil and sediment.

Based on modelled ultimate degradation data (Table 4) and expert judgment (Danish EPA 1999, ETAD 1995), Solvent Red 23 meets the persistence criteria in water, soil and sediment (half-lives in aerobic soil and water ≥182 days and half-life in aerobic sediment ≥365 days) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential for Bioaccumulation

No experimental bioaccumulation data are available for Solvent Red 23. Since bioaccumulation models are known to predict poorly for pigments and dyes, predictions from such models are considered unreliable for disazo dyes. As a result, bioaccumulation modelling has not been used to evaluate the bioaccumulation status of these substances.

In the absence of experimental and modelled data, empirical bioconcentration (BCF) and bioaccumulation (BAF) factors for structural analogues were used to estimate the bioaccumulation potential of Solvent Red 23. To that end, bioconcentration studies for relatively close structural analogues Sudan IV (MITI 1992) and Disperse Orange 30 (Shen and Hu 2008), suggest that Solvent Red 23 is unlikely to accumulate in fish.

The test on Solvent Red 23 (Table 5a) performed by the Japanese Ministry of International Trade and Industry (MITI) using common carp resulted in a series of low bioconcentration factors less than 11 L/kg.

Table 5a. Empirical data for bioaccumulation and bioconcentration of Sudan IV, an analogue of Solvent Red 23

Test Organism	Experimental Concentration (mg/L) and/or Exposure Source	Endpoint (BCF, L/kg)	Reference
Common carp (*Cyprinus carpio*)	0.35	<0.29-2.9	MITI 1992
Common carp (*Cyprinus carpio*)	0.035	<2.9-11	MITI 1992

The bioconcentration test by Shen and Hu (2008) was performed according to OECD Guidelines (OECD 1996). The bioconcentration of Disperse Orange 30 in zebra fish (*Brachydanio rerio*) was determined in a 28-day semi-static test with test medium renewal every two days. An exposure test at a nominal concentration of 20 mg/L (mean measured concentration 0.028 - 0.28 mg/L) was performed in accordance with the result of the fish acute toxicity test to check the bioconcentration potential of the test substance.

Samples from both test solutions and test organisms were taken daily from Day 26 to Day 28 during the 28-day exposure test period. Samples were prepared by extracting the lipid component from the test fish. The measured concentration of test substance, fish lipid content and BCF calculation are reported in Table 5b.

Table 5b. Measured concentrations, fish lipid content and BCF calculation for analogue Disperse Orange 30

		Sampling Time		
		Day 26	**Day 27**	**Day 28**
Treatments (20 mg/L)	Measured concentration of the test substance in extracted solutions (mg/L)	<0.028	<0.028	<0.028
	Content of the test substance in the fish lipids (mg)	<1.68	<1.68	<1.68
	Fish total weight (g)	2.07	2.13	2.53
	Concentration of the test substance in the fish C_f (mg/kg)	<0.81	<0.79	<0.66
	Measured concentration of the test substance in the water C_w (mg/L)	0.028 - 0.28	0.028 - 0.28	0.028 - 0.28
	Fish lipid content (%)	0.81	0.57	1.25
	BCF	<100	<100	<100
	Average BCF	<100		

The Shen and Hu (2008) study has been reviewed and considered acceptable (see Appendix 1). The very low level of detection in fish extracts (<0.028 mg/L) suggests a limited solubility in lipids and/or limited potential to partition into fish tissue from aqueous systems. However, there is some uncertainty associated with limit-bounded values in any study because the actual value is not known. But given the structure and likely behavior of disperse dyes in aqueous systems, a low BCF result is expected. Most disperse dyes, as their name suggests, exist as fine dispersible particles with limited truly soluble fractions. Solubility, however, can be increased by adding polar functional groups to the molecule. While Solvent Red 23 and relevant analogues (Table 2) contain some of these solubilizing functional groups (phenol groups), relevant experimental solubility values for Solvent Red 23 and relevant analogues (i.e., 0.00006–0.345 mg/L) are relatively low – being below or comparable to (less than one order of magnitude above) the water solubility for Disperse Orange 30 (i.e., 0.07 mg/L). As a result, it is expected that Solvent Red 23 would have a bioavailability and bioconcentration potential that is similar to or lower than that of Disperse Orange 30.

While the above studies serve as primary evidence to indicate the lack of bioaccumulation potential for Solvent Red 23, other research corroborates this conclusion. Anliker et al. (1981) reported experimental fish bioaccumulation values for 18 monoazo disperse dyes, performed according to test methods specified by MITI. Expressed on the basis of wet body weight of the fishes, these log bioaccumulation factors ranged from 0.00 to 1.76 (Anliker et al. 1981). A lack of reporting of chemical

registry numbers and chemical structures limited the utility of this study for read-across purposes to Solvent Red 23. However, follow-up studies, which provided the chemical structures for the disperse dyes tested, confirmed low bioaccumulation potential for ten nitro-substituted azo dyes, with reported log bioaccumulation factors ranging from 0.3 to 1.76 (Anliker and Moser 1987; Anliker et al. 1988). Studies available from MITI also support low bioaccumulation potential for disperse azo dyes. Reported BCFs for 3 disperse azo dyes (CAS Nos. 40690-89-9, 61968-52-3 and 71767-67-4) tested at a concentration of 0.01 mg/L were in the range of <0.3 to 47 L/kg (MITI 1992). An accumulation study by Brown (1987) also showed that none of the twelve disperse dyes tested accumulated during an eight week study with carp.

High read-across log K_{ow} values for related azo analogues (Table 2) is the only line of evidence that suggests that Solvent Red 23 may have a high potential for bioaccumulation. In spite of the high K_{ow} values for the azo structural analogues, evidence for bioaccumulation of disperse azo dyes is lacking (Anliker et al. 1981; Anliker and Moser 1987; Anliker et al. 1988; MITI 1992). Authors who have measured high log K_{ow}s and concomitant low bioaccumulation factors for disperse azo dyes suggest that the low accumulation factors may be due to their low absolute fat solubility (Brown 1987) or relatively high molecular weight, which may make transport across fish membranes difficult (Anliker et al. 1981; Anliker and Moser 1987). It is also likely that the lack of bioavailability and limited capacity to partition under BCF test conditions limits accumulation in fish lipids.

Recent investigations relating fish BCF data and molecular size parameters (Dimitrov et al. 2002, 2005) suggest that the probability of a molecule crossing cell membranes as a result of passive diffusion declines significantly with increasing maximum diameter (D_{max}). The probability of passive diffusion decreases appreciably when the maximum diameter is greater than ~1.5 nm and much more so for molecules having a maximum diameter of greater than 1.7 nm. Sakuratani et al. (2008) have also investigated the effect of cross-sectional diameter on passive diffusion in a BCF test set of about 1200 new and existing chemicals. They observed that substances that do not have a very high bioconcentration potential (BCF <5000) often have a D_{max} of >2.0 nm and an effective diameter (D_{eff}) >1.1 nm.

However, as Arnot et al. (2010) have noted, there are uncertainties associated with the thresholds proposed by Dimitrov et al. (2002, 2005) and Sakuratani et al. (2008) since the BCF studies used to derive them were not critically evaluated. Arnot et al. (2010) point out that molecular size influences solubility and diffusivity in water and organic phases (membranes), and larger molecules may have slower uptake rates. However, these same kinetic constraints apply to diffusive routes of chemical elimination (i.e., slow in = slow out). Thus, significant bioaccumulation potential may remain for substances that are subject to slow absorption processes, if they are slowly biotransformed or slowly eliminated by other processes. Consequently, when evaluating bioaccumulation potential, molecular size information should be considered with care and used together with other relevant lines of evidence in a weight-of-evidence approach.

Solvent Red 23 and its closest analogues (the disazo dyes) have molecular weights ranging between 302 and 377 g/mol (see Table 3b) and their molecular structures are relatively uncomplicated; both these characteristics indicate a bioaccumulation capability of these substances if molecular weight is used as the only indicator. In addition, Arnot et al. (2010) points out that there are no clear relationships for establishing strict molecular size cut-offs for assessing bioaccumulation potential. However, the report does not dispute the notion that a reduction in uptake rate can be associated with increasing cross-sectional diameter as demonstrated by Dimitrov et al. (2002, 2005). The maximum diameter of Solvent Red 23, its closest analogues and their conformers ranges from 1.5 to 2.2 nm (BBM 2008), suggesting that a potential for a significantly reduced uptake rate from water and reduced in vivo bioavailability exists with these dyes.

Based on a lack of accumulation observed in bioconcentration tests with Sudan IV, Disperse Orange 30 and other related disperse azo dyes that showed similar results, as well as data showing large cross-sectional diameters for Solvent Red 23 and its analogues that likely limit their partitioning behavior, Solvent Red 23 is expected to have a low potential for bioaccumulation. Therefore, considering the available evidence, Solvent Red 23 does not meet the bioaccumulation criteria (BCF or BAF $\geq$5000) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential to Cause Ecological Harm

Ecological Effects Assessment

A - In the Aquatic Compartment

Toxicity studies were not submitted for Solvent Red 23. However, read-across data were submitted for several analogues of Solvent Red 23 including studies on Disperse Yellow 23 and Disperse Orange 29 (Study Submission 2008a,b). According to these studies, Sudan IV has a 48-hour LC_{50} of >100 mg/L in Japanese killifish (*Oryzias latipes*). Disperse Yellow 23 has a 48-hour LC_{50} of >1000 mg/L in rainbow trout (*Oncorhynchus mykiss*) and Disperse Orange 29 has a 96-hour LC_{50} of 480 mg/L in zebra fish (*Brachydanio rerio*) (Table 6). In addition, Disperse Orange 29 has a 72-hour EC_{50} of 6 mg/L in algae (*Scenedesmus subspicatus*) and a 48-hour EC_{50} in *Daphnia magna* of 70 mg/L. Due to lack of details, these studies were deemed of uncertain reliability (see Appendix 1). However, these data were considered usable in this screening assessment in a weight-of-evidence context.

In addition, a study submitted on behalf of ETAD provides acute ecotoxicity data in fish, invertebrates, algae and bacteria for 5 nitro-substituted azo disperse dyes (Brown 1992). Acute zebra fish, *Daphnia magna* and *Scenedesmus subspicatus* toxicity for the 5 analogues ranged from 17 to 710 mg/L, from 4.5 to 110 mg/L and from 6.7 to 54 mg/L, respectively (Table 6). In addition, all bacteria tests had an IC_{50} exceeding 100 mg/L. The experimental details for the dyes tested were not provided, which greatly limited

evaluation of these studies (Brown 1992). However, these data were considered usable and are included in this screening assessment as part of the weight of evidence.

Another acute fish toxicity study was submitted for the analogue Disperse Blue 79 (BASF 1990). According to the study, Disperse Blue 79 has a 96-hour LC_{50} in golden orfe between 100 and 220 mg/L (Table 6). However, due to lack of details, this study was also considered of uncertain reliability (Appendix 1). A fish toxicity study on analogue Sudan IV of >100 mg/L (MITI 1992) was also included in Table 6 to contribute to the weight of evidence but was not preferred as a critical value since the endpoint is not bounded.

Ecotoxicological data for another disperse azo dye were received through the *New Substances Notification Regulations* (Environment Canada 1995). An acute fish toxicity study submitted to meet notification requirements revealed this substance has a 96-hour LC_{50} of 505 mg/L in rainbow trout (Table 6). The test was conducted according to OECD guideline No. 203. The Material Safety Data Sheet provided as part of this notification also contained information on bacterial toxic effects. The results indicate an activated sludge respiration inhibition EC_{50} of >1000 mg/L. Based on the available ecotoxicity information, the new substance was considered to be of low concern for toxic effects to aquatic organisms. Reliability of the study was assessed using a robust study summary and considered as satisfactory (Appendix 1).

Lastly, a chronic study submitted for the analogue Disperse Blue 79:1, revealed its 122 day no-observed-effect concentration (NOEC) in rainbow trout to be greater than 0.0048 mg/L (Table 6). Reliability of this study was assessed as high (Appendix 1). However, this value was not used to calculate the predicted no-effect concentration (PNEC) because the value is an unbounded result (i.e., no certainty as to the threshold for effects).

When considering all structural analogue toxicity information in concert with the toxicity values for Disperse Yellow 23 and Disperse Orange 29, these data suggest that Solvent Red 23 is not highly hazardous to aquatic organisms (i.e., acute LC_{50} values are >1 mg/L).

Table 6. Empirical aquatic toxicity data for analogues of Solvent Red 23

Common name or (CAS RN)	Test organism	Severity (Duration)	Endpoint	Value (mg/L)	Reference
Sudan IV (85-83-6)	*Oryzias latipes*	Acute (48 hours)	LC_{50}[1]	>100	MITI 1992
Disperse Orange 29 (19800-42-1)	Algae	Chronic (72 hours)	EC_{50}[2]	6	Study Submission 2008a
	Daphnia	Acute (48 hours)	EC_{50}	70	
	Fish	Acute (96 hours)	LC_{50}	480	
Disperse Yellow 23 (6250-23-3)	Fish	Acute (48 hours)	LC_{50}	>1000	Study Submission 2008b

Disperse Blue 79[3] (12239-34-8)	Golden orfe	Acute (96 hours)	LC_{50}	100< LC_{50} <220	BASF 1990
	Zebra fish	Acute (96 hours)	LC_{50}	340	Brown 1992
	Daphnia magna	Acute (48 hours)	EC_{50}[3]	4.5*	
	Scenedesmus subspicatus	Chronic - growth (72 hours)	EC_{50}	9.5	
	Bacteria	Not available	IC_{50}[4]	>100	
Disperse Red 73[5] (16889-10-4)	Zebra fish	Acute (96 hours)	LC_{50}	17	
	Daphnia magna	Acute (48 hours)	EC_{50}	23	
	Scenedesmus subspicatus	Chronic - growth (72 hours)	EC_{50}	>10	
	Bacteria	Not available	IC_{50}	>100	
Disperse Orange 30[6] (5261-31-4)	Zebra fish	Acute (96 hours)	LC_{50}	710	
	Daphnia magna	Acute (48 hours)	EC_{50}	5.8	
	Scenedesmus subspicatus	Chronic - growth (72 hours)	EC_{50}	6.7	
	Bacteria	Not available	IC_{50}	>100	
Disperse Orange 25[7] (31482-56-1)	Zebra fish	Acute (96 hours)	IC_{50}	268	
	Daphnia magna	Acute (48 hours)	LC_{50}	110	
	Scenedesmus subspicatus	Chronic - growth (72 hours)	EC_{50}	54	
	Bacteria	Not available	EC_{50}	>100	
Disperse Red 17 (3179-89-3)[8]	Zebra fish	Acute (96 hours)	LC_{50}	103	
	Daphnia magna	Acute (48 hours)	EC_{50}	98	
	Scenedesmus subspicatus	Chronic - growth (72 hours)	EC_{50}	7	
	Bacteria	Not available	IC_{50}	>100	
Analogue azo disperse dye (CAS# confidential)	Rainbow trout	Acute (96 hours)	LC_{50}	505	Environment Canada 1995
Disperse Blue 79:1 (3618-72-2)	Rainbow trout	Chronic (122 days)	NOEC[9]	>0.0048	Cohle and Mihalik 1991

[1] LC_{50} − The concentration of a substance that is estimated to be lethal to 50% of the test organisms.
[2] EC_{50} − The concentration of a substance that is estimated to cause some toxic sublethal effect on 50% of the test organisms.
[3] The study indicates that the Disperse Blue 79 used in the test had a purity (as organic materials) of 76% and a dispersion of 20% dye stuff.

[4] IC_{50} – The inhibiting concentration for a specified percent effect. A point estimate of the concentration of a test substance that causes a 50% reduction in a quantitative biological measurement such as growth rate.
[5] The study indicates that the Disperse Red 73 used in the test had a purity of 96.6%.
[6] The study indicates that the Disperse Orange 30 used in the test had a purity (as organic materials) of 73% and a dispersion of 20% dye stuff.
[7] The study indicates that the Disperse Orange 25 used in the test had a purity of 94%.
[8] The study indicates that the Disperse Red 17 used in the test had a purity of 98.8%.
[9] NOEC – The no-observed-effect concentration is the highest concentration in a toxicity test not causing a statistically significant effect in comparison to the controls.
[*]The critical toxicity value used to derive a probable no effect concentration.

In general, due to their poor solubility (i.e., <1 mg/L), disperse dyes are expected to have a low acute ecological impact (Hunger 2003). The results of empirical toxicity studies with analogues of Solvent Red 23 are consistent with this expectation, indicating fish LC_{50} values in the 17-505 mg/L range, with *Daphnia* being the most sensitive organism tested (EC_{50}/LC_{50}s from 4.5 to 110 mg/L). The critical value chosen to derive a probable no effect concentration was deemed to be the *Daphnia* magna EC_{50} value of 4.5 mg/L (Brown 1992).

Although interpretation of results from these tests is complicated by the fact that the reported effect values (i.e., EC_{50} and LC_{50}s) are likely to be much greater than the solubility of the substances tested. In effect, some of the concentrations reported in Table 6 may represent the loading levels of the test substance. Thus, a subset of the actual LC_{50} or EC_{50} values may be lower than the level reported, as the actual concentration dissolved in water that may cause an effect is not known. In other cases (see footnotes of Table 6), substances tested were in formulations and so were not 100% pure. Therefore, other chemicals in the formulation may have increased solubility and also contributed to the total toxicity. Despite uncertainties in regards to water solubility and the purity of some analogues, the experimental and analogue data available do indicate that the toxicity of Solvent Red 23 is likely to be low.

A range of aquatic toxicity predictions for Solvent Red 23 were also obtained from various QSAR models. However, as with bioaccumulation, the QSAR ecotoxicity predictions for these substances are not considered reliable because of the potential error associated with input parameters and the unique nature of disperse dyes—specifically physical state, structural and/or physical and chemical properties which fall outside of the models' domain of applicability.

The available empirical ecotoxicity information for analogues of Solvent Red 23 suggests that it is not likely to be highly hazardous to aquatic organisms.

B - In Other Environmental Compartments

Because Solvent Red 23 is expected to accumulate in sediment and could be released to soil from direct application of pesticides, the land application of biosolids which is commonly used for soil enrichment, and from the landfill disposal of products that degrade and release these substances, it would be desirable to have toxicity data for sediment and soil organisms. However, no suitable ecological effects studies were found for these compounds or their analogues in media other than water. Considering the lack

of bioaccumulation potential and bioavailability as well as the physical and chemical properties of Solvent Red 23, the toxicity potential is also likely to be low in sediment- and soil-dwelling species. However, this assumption cannot be substantiated due to lack of suitable whole organism toxicity data.

Ecological Exposure Assessment

No data concerning concentrations of Solvent Red 23 in water in Canada have been identified. Environmental concentrations are therefore estimated from available information, including substance quantities, estimated release rates, and characteristics of receiving water bodies.

A – Industrial Release

Solvent Red 23 was not reported to be manufactured or processed in Canada based on industrial submissions for 2005 and 2006; therefore no industrial releases in Canada are expected.

B – Consumer Release

Based on its use pattern in cosmetic and some personal care products, it is anticipated that products containing Solvent Red 23 could be released to sewer or surface water during their use. Therefore, Environment Canada's spreadsheet model to estimate down-the-drain releases from consumer uses (Mega Flush) was employed to estimate the potential substance concentration in multiple water bodies receiving STP effluents to which consumer products containing these substances may have been released (Environment Canada 2008b). The spreadsheet tool provides these estimates for approximately 1000 release sites across Canada based on conservative assumptions.

The conservative assumptions include:

- loss to sewer at 100%,
- STP removal rate estimated at 0.0%,
- number of annual release days at 365 days/year,
- receiving water dilution factor in the range of 1 to 10.

The predicted environmental concentrations (PECs) of Solvent Red 23 in the receiving water bodies were estimated to be in the range of 6.7×10^{-6} to 0.0061 mg/L. The estimate is based on a total of 4000 kg/year for the quantity of the substance based on the upper reporting range of Solvent Red 23 that was reportedly imported in Canada in 2005 from the section 71 survey (Environment Canada 2006). The equation and inputs used to calculate the PEC are described in Environment Canada (2010).

Solvent Red 23 is also used as a formulant in agricultural pesticides (PMRA 2010) and is specifically on the Pest Management Regulatory Agency (PMRA) List 2 which contains formulants that are considered potentially toxic, based on structural similarity to List 1 formulants (which are those described by PMRA as being of significant concern) or on

data suggestive of toxicity. Solvent Red 23 is not expected to be released to sewers through its use in pesticides, but would be released to the environment though agricultural applications of the chemical. This usage is not modeled as pest control products and their formulants are regulated in Canada by the PMRA.

Characterization of Ecological Risk

The approach taken in this ecological screening assessment was to examine available scientific information and develop conclusions based on a weight-of-evidence approach and precaution as required under CEPA 1999.

Based on read-across physical and chemical properties, Solvent Red 23 is predicted to degrade slowly in the aerobic environment and is expected to be persistent in water, soil and sediment. This substance is expected to have low bioaccumulation potential. Although the proportion of Solvent Red 23 that is expected to be released into sewers is high, the low importation quantities of Solvent Red 23 into Canada, along with information on physical and chemical properties, indicate a low potential for overall releases into the Canadian environment. If released into the environment, this substance is expected to be discharged mainly to waste waters, although it is expected to ultimately be transferred to sediment and soil. Through use of analogue data, Solvent Red 23 has also been demonstrated to have only a moderate potential for acute toxicity to aquatic organisms.

A predicted no-effect concentration (PNEC) was estimated based on the 48-hour EC_{50} of 4.5 mg/L in *Daphnia magna* for analogue Disperse Blue 79 (Table 6). A factor of 100 was then applied to account for acute to chronic toxicity and lab to field extrapolations and use of a surrogate substance. The resulting PNEC is 0.045 mg/L.

A risk quotient analysis, integrating a conservative PEC with a conservative estimate of the potential to cause adverse effects, or PNEC, was conducted for the aquatic environment and the resulting risk quotient (PEC/PNEC) is an important line of evidence in evaluating the potential risk to the environment.

For exposure resulting from down-the-drain releases through consumer uses (conservative scenario), the maximum PEC for Solvent Red 23 resulting from Mega Flush was 0.0061 mg/L. When the maximum PEC is compared to the PNEC of 0.045 mg/L, the risk quotient (PEC/PNEC) is 0.136. The risk quotients are <1 at all of the Megaflush sites. This indicates that down-the-drain consumer releases of Solvent Red 23 are not expected to harm aquatic organisms.

Therefore Solvent Red 23 is unlikely to be causing harm to populations of aquatic organisms in Canada.

Uncertainties in Evaluation of Ecological Risk

The persistence assessment is limited by the absence of biodegradation data, which necessitated generation of model predictions. Although all model prediction has some degree of error, the aerobic biodegradation model outputs confirmed the expected persistence of Solvent Red 23 given its use and structural characteristics. In addition, the persistence assessment is limited by the uncertainty about the rate and extent to which degradation occurs in anaerobic sediments and whether the degradation products (e.g., aromatic amines) would be biologically available. Although the degradation products are expected to be of limited biological availability because they are expected to form only in relatively deep anoxic sediment, there is still potential for sediment perturbation. Therefore, this issue is a source of uncertainty in the toxicity assessment of Solvent Red 23.

The bioaccumulation assessment for these substances was limited by the lack of empirical data on Solvent Red 23 and the inability of available models to reliably estimate bioaccumulation for disazo dyes. Instead the assessment relied on the use of bioaccumulation data for a structural analogue (Disperse Orange 30).

Uncertainties are also present due to the lack of information on environmental concentrations in Canada for Solvent Red 23. However the lack of reports of manufacturing in Canada, low import quantities and the anticipated removal from the effluent suggests low potential for releases of these chemicals into the Canadian aquatic environment.

Uncertainties are also associated with the fraction of these substances that is released, and with the fraction that is removed in STPs. Those uncertainties were addressed through the use of conservative assumptions in the exposure modelling.

The experimental concentrations associated with toxicity for aquatic organisms may have an additional source of uncertainty when these concentrations exceed the solubility of the chemical in water (either experimental or predicted). There is also some uncertainty related to the purity of the substances used in the solubility and toxicity tests. Due to the low solubility of dyes, often they are mixed with water and a solubilising agent in order to complete the test. This convention can produce solubility values that are artificially high and may affect the result of aquatic toxicity tests as well. However, efforts to make the colourants more soluble are likely to artificially increase bioavailability rather than decrease it. Therefore, despite these uncertainties, the available data indicate that Solvent Red 23 and its analogues are not highly hazardous to aquatic organisms in the water column.

Also, regarding ecotoxicity, based on the predicted partitioning behaviour of Solvent Red 23 and its analogues, the significance of soil and sediment as important media of exposure is not well addressed by the effects data available. Indeed, the only effects data identified apply primarily to pelagic aquatic exposures, although the water column may not be the medium of primary long-term concern based on partitioning estimates and release patterns.

Given the use of this substance in other countries, it is possible that the substance is entering the Canadian market as a component of manufactured items and/or consumer products. Information obtained from the section 71 survey and other information sources indicated that it may be present in a limited number of these types of products in Canada. Available information is currently not sufficient to derive a quantitative estimate to help determine the importance of this source in ecological assessment. However, it is anticipated that the proportions of Solvent Red 23 released to the various environmental media would not be significantly different from those estimated here, although quantities transferred to recycling and/or waste disposal may be higher. It is also recognized that releases from waste disposal sites are possible, although there is a low potential for this to occur and the releases would be likely be low in quantity.

Potential to Cause Harm to Human Health

Exposure Assessment

No empirical data were identified for Solvent Red 23 in environmental media. There was insufficient information on which to base a life cycle analysis to predict loss percentages to estimate environmental concentrations. Therefore environmental concentrations of Solvent Red 23 were estimated based on its vapour pressure and water solubility (lowest of the read across range) and assuming that the upper reporting range of 4000 kg (1000 kg from each of 4 companies reporting) was all released to sewer, wastewater and land. The predicted concentrations in environmental media using ChemCAN Version 6 software are found to be negligible (Environment Canada 2008a; ChemCAN 2003).

Potential exposure to Solvent Red 23 of the general population from the use of cosmetic and some personal care products were estimated using ConsExpo 4.1 (ConsExpo 2006; RIVM 2006). Estimates of dermal exposures from representative cosmetic and personal care products containing Solvent Red 23, such as bath preparation, massage oil, hair-conditioner, hair-dye, body lotion, facial cleanser and nail polish, were derived. Oral exposure was estimated for use of lip-gloss/ lip balm containing Solvent Red 23.

Dermal exposure from use of personal care products was estimated to range from 0.002 mg/kg-bw per day (massage oil) to 0.18 mg/kg-bw per day (body lotion) with a conservative assumption that 26%[1] of the applied product is absorbed. Oral exposure of 0.01 mg/kg bw per day for children aged 5-11 years and 0.006 mg/kg-bw per day for general population were estimated from the use of lip-gloss/lip balm by applying a conservative assumption that 100%[1] of the applied product is ingested. Given the conservative nature of the modelling assumptions and conservatively assuming 26% and 100% absorption via dermal and oral routes, respectively, these values are considered to be upper-bounding estimates of exposure. Exposure to consumer products via inhalation route was considered to be minimal based on the low volatility of this substance.

[1] see Absorption and Bioavailability for information on the basis of the oral and dermal absorption values used for estimating exposure to Solvent Red 23

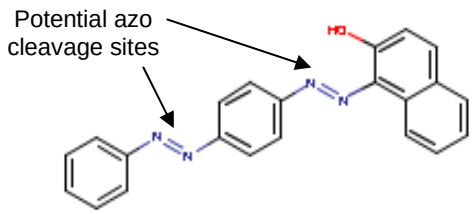
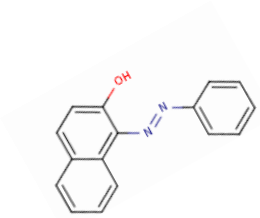
Health Effects Assessment

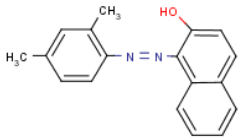
Solvent Red 23 is a member of the family of azo colourants of which many have been demonstrated to undergo reductive cleavage mediated by azoreductase enzymes present in mammalian tissues as well as in bacteria of the intestine and skin (e.g. Chadwick et al 1992; Platzek 1999; Golka et al. 2004; Chen 2006; Stingley et al. 2010). As such, health effects data for potential products of azo cleavage have been considered in this assessment. Analogue data has also been considered in order to inform the health effects database and to shed light on the potential for Solvent Red 23 to undergo azo reductive cleavage. Analogues were selected based on the availability of health effects data and on their structural similarity to Solvent Red 23. Table 7 lists the analogues considered in this assessment as well as the potential azo reductive cleavage products of Solvent Red 23.

Table 7: Analogues and potential azo cleavage products considered for Solvent Red 23 in the human health effects assessment

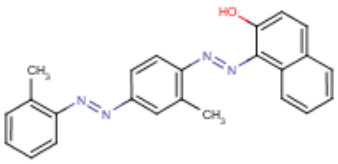
<table>
<tr><th colspan="4">Solvent Red 23</th></tr>
<tr><td colspan="2">Parent Compound:

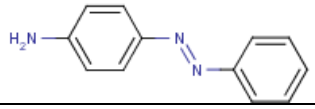
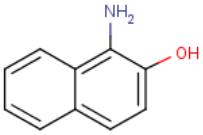
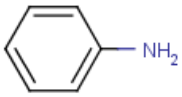
Solvent Red 23 (Sudan III)
(85-86-9)
2-Naphthalenol, 1-[[4-(phenylazo)phenyl]azo]

- disazo, contains hydroxylated naphthalene ring.</td><td colspan="2"> 
</td></tr>
<tr><th colspan="4">Solvent Red 23 Analogues*</th></tr>
<tr><th>Common name (CAS RN)</th><th>DSL name</th><th>Structure</th><th>Major structural similarities and differences with Solvent Red 23</th></tr>
<tr><td>Sudan I / C.I. Solvent Yellow 14 (842-07-9)</td><td>2-Naphthalenol, 1-(phenylazo)-</td><td> 
</td><td>Similarity: contains hyodroxylated naphthalene ring and an azo linkage.

Differences: monoazo, only one phenyl group.</td></tr>
<tr><td>Sudan II / Solvent Orange 7 (3118-97-6)</td><td>2-Naphthalenol, 1-[(2,4-dimethylphenyl)azo]-</td><td> 
</td><td>Similarity: contains hyodroxylated naphthalene ring and an azo linkage.

Differences: monoazo, only one phenyl group which is dimethylated,.</td></tr>
</table>

Sudan IV / Solvent Red 24 (85-83-6)	2-Naphthalenol, 1-[[2-methyl-4-[(2-methylphenyl)azo]phenyl]azo]-		Similarity: disazo, contains hydroxylated naphthalene ring. Differences: Both phenyl rings have one methyl group each on *ortho*- position.

Potential Azo Cleavage Products*	
Potential Azo Cleavage Product	**Structure**
4-aminoazobenzene (60-09-3)	
1-amino-2-naphthol (2834-92-6)	
p-phenylenediamine (106-50-3)	
Aniline (62-53-3)	

* More detailed physical-chemical properties and health effects information is presented in Appendices 6 and 7 for the selected analogues of Solvent Red 23 and the potential cleavage products, respectively.

Potential for Azo Bond Cleavage

Investigations into the ability of human gut bacteria to metabolize Solvent Red 23, resulting in the release of aromatic amines, were performed by incubating Solvent Red 23 with fresh human fecal suspensions or with *Lactobacillus acidophilus* and *Lactobacillus fermentum*. Both studies identified aniline as a metabolite of Solvent Red 23 indicating that human gut microflora are capable of reducing the azo bond of Solvent Red 23 (Xu et al. 2007; Chen et al. 2009). Rats injected intraperitoneally (IP) with radioactively labelled Solvent Red 23 excreted 15.8% of the total administered radioactivity in the urine of which 80% was identified as 4-aminophenol, a hydroxylation metabolite of aniline (Ryan and Welling 1967), demonstrating that azo reductive cleavage of Solvent Red 23 occurs *in vivo*.

Sudan I (CAS RN 842-07-9), a monoazo analogue of Solvent Red 23, was also metabolized to 4-aminophenol and its conjugates in rabbits exposed orally indicating that the two substances share similar metabolic fates that lead to the release of aniline, which is then converted to 4-aminophenol (EFSA 2005). On this basis, EFSA considered it prudent to assume that the genotoxicity and carcinogenicity associated with Sudan I may also be associated with Solvent Red 23 (EFSA 2005).

Sudan IV is a lipophilic disazo methylated analogue of Solvent Red 23, and has also been demonstrated to undergo azo cleavage both *in vivo* and *in vitro*. In the same study that

examined azo reduction of Solvent Red 23, Sudan IV was observed to undergo reductive cleavage when incubated with freshly isolated human fecal suspensions (Xu et al. 2007). Sudan IV was also shown to be metabolically reduced *in vivo* following intratracheal administration in rats, whereby *o*-aminoazotoluene, the dimethyl analogue of 4-aminoazobenzene (a potential azo cleavage product of Solvent Red 23), was detectable in the urine (Parent and Dressler 1977). While the intratracheal route of administration is not relevant to human exposure, the aforementioned study provides evidence that a lipophilic solvent dye, Sudan IV, can be cleaved *in vivo* to release a carcinogenic aromatic amine (*o*-aminoazotoluene) that is very similar in structure and toxicity profile to 4-aminoazobenzene, a predicted azo cleavage product of Solvent Red 23.

Collectively, the data for Solvent Red 23 and its analogues support the potential for reductive cleavage of the azo bonds, particularly when administered by the oral route, and therefore the potential for exposure to aromatic amines. While aniline (CAS RN 62-53-3) was the predominant metabolite detected in studies designed to determine whether the azo bonds of Solvent Red 23 can be metabolically cleaved, the presence of other potential cleavage products cannot be precluded. Consequently, this screening assessment also considers health effects data for the potential azo cleavage products of Solvent Red 23: 4-aminoazobenzene (CAS RN 60-09-3), 1-amino-2-napthol (CAS RN 2834-92-6), and *p*-phenylenediamine (CAS RN 106-50-3). Information on the potential products of azo reductive cleavage are presented in Appendix 6 and summarized here in the Health Effects section.

Solvent Red 23

Although limited empirical data are available, the health effects of Solvent Red 23 have been previously evaluated in several reviews prepared by the International Agency for Research on Cancer, IARC (IARC 1975, 1987), the European Commission Scientific Committee on Consumer Products, SCCP (SCCP 2005, formerly SCCNFP 2002), and the European Food Safety Authority, EFSA (EFSA 2005). A summary of the relevant data from these reviews is presented below along with additional information in Appendix 4. In addition, relevant information from (quantitative) structural activity relationship [(Q)SAR] modelling predictions are presented to inform the health effects assessment for Solvent Red 23 (Appendix 7).

Genotoxicity and Carcinogenicity

Several oral and dermal repeated-dose studies were identified for Solvent Red 23. IARC (1975) cited two chronic oral dietary studies in mice and rats. "Type B" mice (83 males, 54 females) originating from a cross of multiple strains were exposed to Solvent Red 23 (reported as "Sudan G") in the diet at approximately 67 mg/kg-bw/day[1] for up to 752 days (Waterman and Lignac 1958). At the end of the observation period, the authors reported lung tumours in the male (9) and female (7) exposure groups, which were

[1] Approximate dose = 67mg/kg-bw/day based on 2 mg/animal/day reported and mouse body weight of 0.05kg (Health Canada 1994).

statistically greater than in the control groups (0 in males, 1 in females). The authors considered the high lung tumour incidence in exposed mice to be normal for the Type B mouse strain, and a low incidence of lung tumours in controls to be due to non-uniform distribution of mice with different sensitivities. Similar incidences of forestomach papillomas and mammary tumours were observed in control and exposed animals. A report of Wistar strain rats (5/sex) fed Solvent Red 23 (as Sudan III) at 4% in the diet for up to 18 months (approximately 2000 mg/kg-bw/day[1]) did not report any exposure-related effects; however, the gross and microscopic examination appeared to be restricted to the gastrointestinal tract and liver (Willheim and Ivy 1953). IARC considered these data inadequate due to either "the doses administered, the degree of reporting or the number of animals used". Other oral studies not considered by IARC have also been identified. In an earlier study, Solvent Red 23 was administered to rats, via the diet, at a concentration of 0.04% (approximately 21 mg/kg-bw/day[2]) for eleven months. Unspecified kidney changes were reported, but no tumours were observed (Maruya 1938). No tumours were observed in the liver or elsewhere when 16 Long-Evans female rats were fed 0.1% Solvent Red 23 in 1 mL sesame oil (approximately 3mg/kg-bw/day[3]) by gastric instillation for 25 weeks (5x/week) (Huggins et al. 1978). The same authors demonstrated that oral Solvent Red 23, administered to rats at the same dose range, was protective against leukemia and mammary tumours induced by the highly potent carcinogens 7,12-di- and 7,8,12-tri-methylbenz[a]anthracene (DMBA and TMBA, respectively) (Huggins and Pataki 1965, Huggins et al. 1978). A mechanism for this protective effect has been hypothesized to be based on a strong induction, by Solvent Red 23, of Phase I and II enzyme systems that may act to effectively detoxify DMBA and TMBA, *in vivo* (Hatakeyama et al. 1996; Fujita et al. 1988). The studies summarised here add little to the assessment of carcinogenic effects due to their limitations (e.g. single dose exposures, short exposure time etc.).

Repeat-dose dermal studies with Solvent Red 23 have also been identified. A short-term unpublished study reported that rabbits were exposed to Solvent Red 23 concentrations up to 1% (daily) in USP white or hydrophilic ointment vehicle for 21 or 90 days (SCC 1988). Exposures were carried out on both intact and abraded skin. The only reported effects were dermal inflammation, thickening and hyperkeratosis, although the SCCP (2005) considered the study inadequate for evaluation as the method was not described. In a chronic skin painting study, Swiss-Webster mice (50/sex) were exposed once weekly on the shaved dorsal skin to a single exposure concentration of either 1% Solvent Red 23, (D&C Red 17) in 0.1 mL aqueous sodium lauryl sulfate (approximate weekly dose of 33 mg/kg-bw[4]) or to the vehicle alone (Carson 1984). Clinical observations were made daily during the exposure period while histological analyses of all tissues and grossly abnormal

[1] Approximate dose = 2000mg/kg-bw/day based on 40000ppm in diet (4%) and dose conversion of 0.05 for rats (Health Canada 1994)

[2] Exposures reported in percentage of diet. Converted to mg/kg-bw/day using Health Canada reference values (Health Canada 1994).

[3] Estimated dose based on: 1 mg/dose (0.1% in 1mL volume) and 0.35kg body weight for rats (Health Canada 1994)

[4] Estimated dose is approximately 33 mg/kg/bw per weekly dose (equivalent to amortized dose of 5 mg/kg-bw/day) based on 1 mg/weekly applied dose (1% in 0.1mL applied) and 0.03kg body weight for mice (Health Canada 1994).

organs were performed at necropsy following 18 months of exposure or as part of interim sacrifice groups. The study authors reported no pathological changes at the exposure site, or in the examined tissues, that could be attributed to D&C Red 17 exposure at the test concentration studied (Carson 1984).

Based on evaluations by the SCCP (2005, previously SCCNFP 2002), the available carcinogenicity data on Solvent Red 23 were considered to be inadequate for the characterization of the carcinogenicity for this substance. IARC determined the overall cancer classification of this substance to be IARC Group 3 (*not classifiable as to carcinogenicity in humans*) based on inadequate animal data and no human epidemiology data (IARC 1975, 1987). Although the available animal studies for Solvent Red 23, administered both orally and topically, demonstrated no evidence for carcinogenicity at the single doses tested, and may have a protective effect against the carcinogenicity of the known carcinogens DMBA and TMBA (Huggins and Pataki 1965, Huggins et al. 1978), these observations, in and of themselves, do not provide sufficient evidence that Solvent Red 23 is not carcinogenic on its own. Due to incomplete reporting of study details, small numbers of animals used, and/or limited number of doses tested, conclusions cannot be drawn concerning the carcinogenicity of Solvent Red 23 based on these data.

Several studies investigated the genotoxic potential of Solvent Red 23. Ames assays, using *Salmonella typhimurium* strains TA98 and TA100, were negative when purified compound was tested. However, for Solvent Red 23, 3 of 5 commercial preparations were mutagenic in the presence of S9. The authors attributed the positive results to the presence of residual 4-aminoazobenzene (Miyagoshi et al. 1985; SCCNFP 2002). Solvent Red 23 was also negative for genotoxicity in bacterial rec-assays (Kada et al., 1972; Mamber et al. 1983). It should be noted that reduction of the azo bond(s) has proven to be necessary to activate many azo dyes and therefore, since standard S9 systems do not efficiently mediate azo bond cleavage, the negative results in these studies are not conclusive as the method of activation may not be appropriate for this class of compounds (Prival and Mitchell 1982; SCCNFP 2002). Incubation of CHO cells in the presence of Solvent Red 23, without metabolic activation, resulted in an increased frequency of chromosome breaks compared to controls (Au and Hsu 1979) while another *in vitro* test for chromosome aberrations in human lymphocytes was reported to be negative (SCCP 2005). The SCCP considered the data on genotoxicity for Solvent Red 23 to be incomplete and that a conclusion on potential genotoxicity could not be made (SCCNFP 2002, SCCP 2005).

The (Q)SAR models, CASETOX, DEREK, and TOPKAT, provided mixed results regarding the genotoxic and carcinogenic potential of Solvent Red 23 as summarized in Appendix 7 (CASETOX 2008; DEREK 2008; TOPKAT 2004).

Non-Cancer Effects

Irritation and sensitization potential of Solvent Red 23 was summarized in reports by SCCP. Solvent Red 23 was considered to be a marginal irritant based on evidence of being slightly irritating to the skin and mucous membranes of rabbits (SCC 1988, SCCP

2005). Investigations of skin sensitization showed that individuals administered one of several azo dyes were not sensitized to Solvent Red 23 (Kozuka and Tashiro 1980; SCCP 2005). However, 3/6 and 1/6 guinea pigs exposed to the aromatic amine *p*-phenylenediamine or 4-aminoazobenzene, respectively, were sensitized to Solvent Red 23 (Xie et al. 2000; SCCP 2005).

Reproductive effects in males via *in utero* exposure were investigated in one oral study in which pregnant mice were exposed by gavage to Solvent Red 23 (1000 mg/kg-bw/day) or vehicle alone on gestational days 8 – 12 (Gray and Ostby 1993). The testes of male offspring (40 from Solvent Red 23 group, 19 from control group) were later examined at 48-50 days of age. No exposure-related changes were observed in body weight, testes weight, number of seminiferous tubules, percent atrophic tubules, or seminal vesicle weight. The authors also reported no maternal toxicity and no other changes in neonatal parameters; however, these data were not shown in the study.

Analogues

Carcinogenicity and genotoxicity

The genotoxic and carcinogenic potentials of related azo dyes, Sudan I, Sudan II, and Sudan IV, have also been considered in this assessment: (see Table 7 above). These substances are also lipophilic solvent dyes of the Sudan class and share in common with Solvent Red 23 similar physical-chemical properties, component aromatic amines, and metabolism (see also Absorption, Distribution, Metabolism, and Excretion). Therefore, the genotoxic and carcinogenic potentials of these substances are considered to help inform the toxicity profile of Solvent Red 23.

Sudan I is a monoazo analogue in the same Sudan dye class as Solvent Red 23 (Sudan III) and both share in common two identical aromatic amine components (aniline and 1-amino-2-naphthol). In chronic feeding studies conducted by the US National Toxicology Program (NTP), Sudan I was positive for oral carcinogenicity in male and female rats (but not mice) based on a dose-dependent increased incidence of neoplastic liver nodules (NTP 1982). This substance was positive for mutagenicity in the mouse lymphoma forward mutation assay and in Ames bacterial assays with and without metabolic activation by rat and hamster liver S9, but was negative for chromosome aberrations *in vitro* and *in vivo* and equivocal for micronuclei *in vivo* (NTP 2010). Sudan I has been classified as a carcinogen and mutagen in Europe under the CLP Regulations[1] (European Commission 2008), and therefore in the absence of a demonstration of adequate safety (SCCP 2001, 2004) the use of this substance in cosmetics has been prohibited in Europe by virtue of listing (as "CI Solvent Yellow 14") on Annex II of the Cosmetics Directive (European Commission 2010).

Sudan II, also referred to as Solvent Orange 7, is a mono-azo analogue that is similar to Sudan I in structure. In contrast to Sudan I, Sudan II is methylated at the 2 and 4 positions

[1] current classification of Carc. 2 and Mut. 2 in (CLP Annex VI Table 3.1), previous classification of Carc. Cat. 3 and Muta. Cat. 3 (CLP Annex VI Table 3.2) (European Commission 2008, ESIS 1995-2010)

around its phenyl ring. Sudan II has been tested in a limited number of *in vitro* tests for bacterial mutagenicity, which were considered to be sufficient evidence that Sudan II is mutagenic in bacteria after metabolic activation (EFSA 2005). Sudan II was negative in the sole test of mammalian genotoxicity, *in vitro* (EFSA 2005). Several studies for carcinogenicity have been carried out in either mice or rats; however, IARC considered these studies inadequate to asses the carcinogenicity of Sudan II (IARC 1975). EFSA, on the other hand, considered a high incidence of bladder tumours in mice receiving Sudan II implants (directly into the bladder) as sufficient to consider Sudan II *possibly carcinogenic* until proven otherwise (EFSA 2005).

Sudan IV is a lipophilic disazo dimethyl analogue in the same Sudan dye class as Solvent Red 23 (Sudan III) and both share in common one similar (*o*-aminoazotoluene[1]) and one identical (1-amino-2-naphthol[2]) component aromatic amine. Sudan IV has not been classified for carcinogenicity or genotoxicity although it has been tested for carcinogenicity in several dated assays evaluated by IARC (1975). Twenty-four rats were exposed subcutaneously to 0.2 ml of a 2% solution of Sudan IV, once a week, for a total of 403 days. Of eight rats surviving the 403 days, 4 developed local sarcomas; however, this study did not include a vehicle (Tween 80) control group (Umeda 1957, 1958). Mice treated orally did not have an increased incidence of tumours above controls although multiple hepatic adenomata were observed in one treated female that were similar to liver tumours observed in positive control mice exposed to *o*-aminoazotoluene; a predicted azo cleavage product of Sudan IV (Waterman and Lignac 1958). No tumours were observed in Wistar rats exposed to 4% Sudan IV in the diet (approximately 2000 mg/kg-bw/day) for up to 18 months. However, 1/4 of the exposed rats surviving to 18 months had cirrhotic changes in the liver and hyperplasia of bile ducts. No such changes were observed in 50 control rats (Willheim and Ivy 1953). Epithelial hyperplasias, but no tumours, were reported to occur in a skin painting study in mice (IARC 1975). On the basis of the inadequate animal cancer data summarized above and the absence of epidemiological data, IARC classified Sudan IV as Group 3 (*not classifiable as to carcinogenicity in humans*) (IARC 1975, 1987). With regards to genotoxicity, Sudan IV was positive in *Salmonella typhimurium* strain TA98 using the modified Prival method to facilitate azo reduction (Zhou et al. 1987). Positive test results were also observed in TA 1537, 1538, and 98 with dithionate reduction (EFSA 2005). Earlier studies, performed without azo reduction, showed that bacterial mutagenicity was due to an impurity in a commercial grade of dye (Miyagoshi et al. 1985). Sudan IV was also positive for virally enhanced cell transformation in Syrian hamster embryo cells (Heidelberger et al. 1983). Based on the limited evidence of genotoxicity, the ability to induce epithelial proliferation, and structural similarity to the carcinogen Sudan I, a scientific panel of EFSA considered Sudan IV as potentially genotoxic and possibly carcinogenic (EFSA 2005). Based on an opinion of the European Commission Scientific Committee on Cosmetology (SCC) which considered there were indications of carcinogenic potential for Sudan IV (as "CI 26105", SCC 1988), its use as a colourant in cosmetics is currently prohibited in Europe by virtue of its listing on Annex II of the Cosmetics Directive

[1] *o*-aminoazotoluene, the predicted azo cleavage product of Sudan IV, is the dimethyl analogue of 4-aminoazobenzene, a predicted metabolite of Solvent Red 23

[2] both Solvent Red 23 and Sudan IV are predicted to generate 1-amino-2-naphthol by azo cleavage

(European Commission 2010). Sudan IV is also currently prohibited from use in cosmetics in Canada (Health Canada 2009).

In summary, empirical test data on the genotoxicity and carcinogenicity of Solvent Red 23 itself are inconclusive. However, based on evidence of genotoxicity and carcinogenicity for its analogue, Sudan I, and aromatic amines expected to be released by azo reductive cleavage (particularly 4-aminobenzene), genotoxicity and carcinogenicity are considered critical effects for risk characterization for Solvent Red 23.

Potential Azo Reductive Cleavage Products

Solvent Red 23 may be cleaved upon exposure to release potentially hazardous aromatic amines (See section: Potential for Azo Bond Cleavage"). As such, health effects associated with the potential products of azo reductive cleavage of Solvent Red 23 are considered below and are summarised in Appendix 5.

4-Aminoazobenzene (CAS RN 60-09-3) is a recognized animal carcinogen causing liver and skin tumours in rats, and was classified by IARC as Group 2B carcinogen (IARC 1975; 1987). In Europe, 4-aminoazobenzene is classified as a carcinogen under the European CLP Regulation (European Commission 2008, ESIS c1995–2009) and is also included in REACH as one of the 22 aromatic amines of concern, which should not be released from azo colourants used in textiles and leather articles which may come into direct and prolonged contact with human skin or oral cavity (REACH Annex XVII Appendix 8) (European Commission 2006). With regards to genotoxicity, 4-aminoazobenzene was predominantly positive for mutagenicity, clastogenicity and DNA damage in a number of genotoxicity assays both *in vivo* and *in vitro* (references cited in BfR 2003; IARC 1987; BIBRA 1989). The dimethyl analogue, *o*-aminoazotoluene (97-56-3), is classified as a carcinogen by IARC (1975, 1987), NTP (2005), European Commission (2008) and is also listed as one of 22 aromatic amines regulated in azo colourants under REACH (European Commission 2006).

Aniline (CAS RN 62-53-3) has been previously evaluated as a priority substance by Health Canada and Environment Canada (Canada 2010) and in a report of the European Union (EU 2004). In chronic dietary exposure studies, species and sex specific splenic tumours were observed in male (but not female) rats at the highest dose tested; no such tumours were observed in male or female mice. Mixed results were observed in assays of genotoxicity with no genotoxicity being observed in the spleen. Aniline has also been shown to induce methaemoglobin formation in acute and repeated-dose exposure studies in rats, dogs, and human volunteers (EU 2004).

1-Amino-2-naphthol (CAS RN 2834-92-6) has not been studied for the carcinogenic potential, and no adequate repeated-dose investigations were identified (BfR 2003). However, an increase in the number of bladder tumours (10/36 vs. 2/56) and squamous metaplasias (18/36 vs. 9/56) was observed when 1-amino-2-napthol was implanted into the bladder in paraffin pellets (Bonser et al. 1956). 1-amino-2-napthol was positive in

bacterial reverse mutation assays using *Salmonella typhimurium* TA100 (Dillon et al. 1994).

p-Phenylenediamine (CAS RN 106-50-3) toxicity was addressed in a risk assessment by the European Commission's Scientific Committee on Consumer Products (SCCP). The SCCP considered *p*-phenylenediamine as having moderate acute oral toxicity and low dermal toxicity and identified *p*-phenylenediamine as a skin sensitizer (SCCP 2006). *p*-Phenylenediamine is currently listed on Health Canada's Cosmetic Ingredient Hotlist (Health Canada 2009), which is the list of ingredients that are intended to be prohibited or restricted for use in cosmetics, including many personal care products. Under Canadian legislation, cosmetics that contain substances that are harmful to the user cannot be sold. *In-vitro* genotoxicity data for *p*-phenylenediamine are mixed, with both negative and positive results in the Ames assay, the mouse lymphoma assay and the micronucleus test assessing mammalian cell clastogenicity. A no-observed-adverse-effect level (NOAEL) of 4 mg/kg-bw per day was derived from a sub-chronic feeding study in mice based on increased liver and kidney weights. Multiple carcinogenicity studies were identified for *p*-phenylenediamine including chronic feeding studies in B6C3F1 mice and F344 rats conducted by the US National Toxicology Program (NTP) in 1979. *p*-Phenylenediamine did not increase the incidence of tumours in exposed animals (SCCP 2006).

Absorption, distribution, metabolism and excretion

Limited information was available to characterize oral and dermal absorption of Solvent Red 23. In an oral rat study, intact Solvent Red 23 was eliminated mainly via the faeces (83.6 - 95% depending on solvent) while the remainder of the administered dose was unaccounted for (Ryan and Welling 1967). More recently, repeat-exposure studies, using a range of doses (3 – 900 mg/kg-bw/day), have demonstrated that Solvent Red 23 significantly modifies protein content and enzyme activity (e.g. CYP1A, glutathione S-transferase, glutathione peroxidase, menadione-reductase, etc.) in the livers and brains of hamsters, mice, and rats exposed orally (Huggins et al. 1978, Hatakeyama et al. 1996, Romero et al. 2000). The enzyme modulating effects following oral exposure provides support for Solvent Red 23 or its potential products of azo cleavage being absorbed and distributed systemically by the oral route. The monoazo analogue of Solvent Red 23, Sudan I, was readily absorbed following oral administration in rats and rabbits and only small amounts were excreted unchanged (IARC 1975). However, due greater molecular size and lower water solubility of Solvent Red 23 compared to Sudan I (Appendix 5), the oral absorption of the parent structure of Solvent Red 23 is expected to be relatively lower. Overall, information on Solvent Red 23 and its analogue Sudan I suggests that there is potential for absorption by the oral route, therefore a conservative default oral uptake fraction of 100% has been used for calculating exposure estimates.

The absorption of Solvent Red 23 was investigated in an *in vitro* dermal absorption study. Viable porcine skin and non-viable human skin were exposed to a commercially available suntan lotion vehicle containing Solvent Red 23. Between 12 – 15 % of the applied dose was retained in the skin (7.3 – 10.7% in stratum corneum, 1.3 – 5.6% in the combined

dermis/epidermis) with less than 1 % of radiolabel recovered in the receptor fluid (Yourick et al. 2007). Another dermal absorption study also reported skin-bound residues of Solvent Red 23 following application to excised rat skin with or without vehicles designed to enhance penetration; however, the substance was not detected in the receptor fluid (Sasaki et al. 1990). Collier et al. (1993) showed that the monoazo analogue Sudan I (5 μg/cm^2 in acetone) was retained in the skin layers at levels similar to those observed for Solvent Red 23. However, in contrast to Solvent Red 23, up to 30 % of applied Sudan I was found in the receptor fluid, with approximately 26% being absorbed by human skin within 24h. Collier et al. (1993) also reported that up to 50% of the receptor fluid label detected was actually due to the presence of azo reduction products, which was measured as the sum of all aromatic amines (mostly aniline) present, including their *N*-oxidation and *N*-acetylation metabolites. Therefore, the absence of Solvent Red 23 in the receptor fluid reported in the above studies may not be definitive evidence for a lack of dermal absorption since it is possible that unlabelled aromatic amines (or their metabolites), generated by azo reductive cleavage, may have been present, but undetected. Additionally, Yourick et al. (2007) washed skin samples with 1% detergent prior to exposure thereby reducing the number of skin bacteria present. Bacteria have been reported to metabolize azo dyes up to 300 times more efficiently than mammals (Collier et al. 1993) and therefore may result in the release of aromatic amines to their component aromatic amines, which would be expected to have greater absorption than their higher molecular-weight parent compounds (Platzek et al. 1999). Consequently, it is not clear whether the absorption of azo cleavage products or their subsequent metabolites is significant after dermal exposure to Solvent Red 23. Given these uncertainties, a conservative dermal absorption value of 26% has been used in this assessment. The 26% dermal uptake is based on physical-chemical properties of Solvent Red 23 which suggests a low dermal penetration as well as empirical dermal penetration data for the monoazo analogue Sudan I (Collier et al. 1993). However, due to greater molecular size and lower water solubility of Solvent Red 23 compared to Sudan I, the dermal absorption of the parent structure of Solvent Red 23 is expected to be relatively lower. Therefore, the selected dermal absorption value of 26% is considered a conservative value for estimating dermal exposure to Solvent Red 23.

Characterization of Risk to Human Health

Exposure of the general population to Solvent Red 23 from environmental media is expected to be negligible. There is no expected exposure to Solvent Red 23 from food as it is not permitted for use in foods intended to be sold in Canada as per Division 16 of the Food and Drugs Regulations, nor has it been identified for use in food packaging. However, dermal and oral exposure from use of cosmetic and some personal care products may occur. The upper-bounding estimate of chronic dermal exposure from individual cosmetic products (including bath preparation, massage oil, hair-conditioner, hair-dye, body lotion, facial cleanser and nail polish) ranges from 0.002 to 0.18 mg/kg-bw per day assuming a conservative dermal absorption value of 26%. Oral exposure to Solvent Red 23 may also occur through the use of lip-gloss/lip balm giving an upper-bounding chronic exposure estimate of 0.01 mg/kg-bw per day for children aged 5-11 years and 0.006 mg/kg-bw per day for the general population. Exposure by the inhalation route from these sources is expected to be minimal.

Empirical health effects data for Solvent Red 23 were limited. Results from genotoxicity assays were mixed. Available carcinogenicity studies were considered inadequate by IARC and the SCCP. Since Solvent Red 23 may undergo azo cleavage, health effects data on the predicted azo cleavage products, particularly 4-aminoazobenzene, have been considered in the health effects characterization for Solvent Red 23. While 4-aminoazobenzene has not been detected in *in vitro* metabolic studies with Solvent Red 23, a closely related analogue, Sudan IV, did undergo azo cleavage *in vivo* to release *o*-aminoazotoluene (methylated analogue of 4-aminoazobenzene). This suggests that 4-aminoazobenzene may also be released from Solvent Red 23 *in vivo*. 4-aminoazobenzene has been shown to induce tumours in rats following oral and dermal exposure, has been classified as a carcinogen by IARC (1987) and the European Commission (2010), and has also demonstrated positive genotoxicity in a range of assays *in vivo* and *in vitro* (references cited in BfR 2003, IARC 1987, BIBRA 1989). In addition, the monoazo analogue dye Sudan I, a classified carcinogen and mutagen by the European Commission, has been shown to be metabolized to some of the same aromatic amines as Solvent Red 23 *in vitro* and *in vivo*, namely aniline and 1-amino-2-napthol.

Based on evidence of genotoxicity and carcinogenicity for Sudan I, an analogue of Solvent Red 23, and based on aromatic amines expected to be released by azo reductive cleavage of Solvent Red 23 (i.e. 4-aminoazobenzene), genotoxicity and carcinogenicity are considered critical effects for risk characterization of Solvent Red 23. Based on consideration of exposure potential to the general population from use of cosmetics and personal care products containing Solvent Red 23 and potential of genotoxicity and carcinogenicity for which there may be a probability of harm at any level of exposure, Solvent Red 23 is concluded to be entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

Uncertainties in Evaluation of Risk to Human Health

There are limited health effects data available for Solvent Red 23 itself, and therefore the hazard profile is based principally on that of potential azo cleavage products and analogue azo dyes. While the available *in vitro* metabolism data for Solvent Red 23 using gastrointestinal tract (GIT) bacteria suggests that there is likely to be azo reductive cleavage following oral exposures, there is uncertainty as to the extent of cleavage as well as the relative proportion and identity of cleavage products produced. Therefore, there is also uncertainty as to the degree to which the general population of Canada may be exposed to the potential azo cleavage products of Solvent Red 23 by the oral route.

There is uncertainty regarding the potential for dermal absorption of Solvent Red 23 and uncertainty as to whether the component aromatic amines will be released upon dermal exposure. A dermal absorption of 26% has been selected based on physical and chemical properties of Solvent Red 23 and empirical data for its analogue, Sudan I. However, this dermal absorption value is considered conservative.

There is a higher degree of confidence that Solvent Red 23 (or its predicted cleavage products) is absorbed via the oral route, due to the observed enzyme induction in multiple species following oral exposures, than via the dermal route. There is also a high degree of confidence that Solvent Red 23 would be cleaved to release aromatic amines after oral exposure as evidenced by azo cleavage by isolated GIT bacteria. Therefore, there is greater relative concern for the oral route of exposure (i.e. lip-gloss/lip-balm) for which a potential hazard is supported by the available data. While a lack of supporting data does not preclude possible risks arising from dermal route exposures, the potential health risks posed by dermal exposure are considered to be lower than those posed by oral route exposures.

Estimates of exposure to Solvent Red 23 via use of cosmetic and some personal care products are considered to be overestimates based on several conservative assumptions such as dermal absorption of 26% and the use of the upper range of reported concentrations in products from the CNS database. However, total exposure to Solvent Red 23 may be underestimated since estimates were not derived for concurrent uses of multiple cosmetic or personal care products. However, there is confidence that exposure to the general population of Canada from these products does not exceed the estimates presented in this assessment.

Conclusion

Based on the information available, it is concluded that Solvent Red 23 is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends. Additionally, Solvent Red 23 meets the criteria for persistence but does not meet the criteria for bioaccumulation potential as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Based on the potential general population exposure, and evidence of genotoxicity and carcinogenicity for 4-aminoazobenzene (potential azo cleavage product) and Sudan I (analogue azo dye), it is concluded that Solvent Red 23 is entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

It is therefore concluded that Solvent Red 23 meets one or more criteria under section 64 of CEPA 1999.

Considerations for Follow-up

Solvent Red 23 belongs to a group of azo substances that may metabolize to aromatic amines, which as a chemical class are known to exhibit hazardous properties, including carcinogenicity. Therefore, additional activity (e.g., research, assessment, monitoring and surveillance) to characterize the risk to human health in Canada of this broader group of azo substances may be undertaken. A Notice of Intent outlining how the Government of Canada will address this group of substances is available at the following internet address: http://www.chemicalsubstanceschimiques.gc.ca/plan/approach-approche/azo_benzidine-eng.php .

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Appendix 1 - Robust Study Summaries for Key Studies

Robust Study Summaries Form and Instructions: Persistence in Water, Sediments and Soil				
No.	**Item**	**Weight**	**Yes/No**	**Specify**
1	Reference: Bio-elimination study for CAS# 6250-23-3 (Disperse Yellow 23) Clariant dyestuff product = Foron Yellow E RGFL (Study Submission 2008b)			
2	Substance identity: CAS RN	n/a[1]	Y	6250-23-3
3	Substance identity: chemical name(s)	n/a	Y	Disperse Yellow 23
4	Chemical composition of the substance	2	N	
5	Chemical purity	1	Y	54% Disperse Yellow 23
Method				
6	Reference	1	N	
7	OECD, EU, national or other standard method?	3	N	
8	Justification of the method/protocol if a nonstandard method was used	2	N	
9	GLP (good laboratory practice)	3		Not applicable (study conducted 1976)
Test design / conditions				
10	Test type (i.e., hydrolysis, biodegradation)	n/a	Y	Biodegradation
11	Test conditions type (aerobic or anaerobic)	n/a	N	Not reported
12	Test medium (water, sediment or soil)	n/a	Y	Water (test medium is inferred as water since test concentration given in mg/L)
13	Test duration	n/a	Y	14 days
14	Negative or positive controls?	1	N	
15	Number of replicates (including controls)	1	N	
16	Measured concentrations reported?	3	N	
17	Analytical method / instrument	1	N	
Details on biodegradation				
18	Type of biodegradation (ready or inherent) reported?	2	N	Not reported
19	When type of biodegradation (ready or inherent) is not reported, is there indirect information that allows for the identification of biodegradation type?	1	N	
20	Inoculum source	1	N	
21	Inoculum concentration or number of microorganisms	1	N	
22	Were inoculum pre-conditioning and pre-adaptation reported?	1	N	
23	Were inoculum pre-conditioning and pre-adaptation appropriate for the method used?	n/a	N	

[1] n/a = not applicable

24	Temperature	1	N	Not reported
25	Has percentage degradation of the reference compound reached the pass levels by Day 14?	n/a	N	No reference compound tested
26	**Soil:** soil moisture reported?	1		
27	**Soil and sediments**: background SOM (soil organic matter) content reported?	1		
28	**Soil and sediments**: clay content reported?	1		
29	**Soil and sediments:** CEC (cation exchange capacity) reported?	1		
	Details on hydrolysis			
30	pH values reported?	1		
31	Temperature	1		
32	Were appropriate concentrations of the substance used?			
33	If solvent was used, was it done appropriately?			
	Details on photodegradation			
34	Temperature	1		
35	Light source	1		
36	Light spectrum (nm)	1		
37	Relative intensity based on sunlight intensity	1		
38	Spectrum of a substance	1		
39	**Indirect photolysis:** sensitizer (type)	1		
40	**Indirect photolysis:** concentration of sensitizer	1		
	Results			
41	Endpoint and value	n/a	n/a	Avg. 14-day biodegradation = 51%
42	Breakdown products	n/a	N	
43	***Score*: .%**		***4.5***	
44	***EC reliability code*:**		***4***	
45	***Reliability category (high, satisfactory, low)*:**		***Not Satisfactory***	
46	***Comments***			

Robust Study Summaries Form: Aquatic B				
No.	**Item**	**Weight**	**Yes/No**	**Specify**
1	**Reference:** Shen G, Hu S. 2008. Bioconcentration Test of C.I. Disperse Orange 30 in Fish. Prepared by Environmental Testing Laboratory, Shanghai Academy of Environmental Sciences, Shanghai, China for Dystar in the name of Ecological and Toxicological Association of the Dyes and Organic Pigments Manufacturers (ETAD) Basel, Switzerland. Report No. S-070-2007. Submitted to Environment Canada in April 2008. Challenge Submission ID#8351			
2	Substance identity: CAS RN	n/a[1]	Y	5261-31-4
3	Substance identity: chemical name(s)	n/a	Y	Propanenitrile, 3-[[2-(acetyloxy)ethyl][4-[(2,6-dichloro-4-nitrophenyl)azo]phenyl]amino]-
4	Chemical composition of the substance	2	N	
5	Chemical purity	1	N	
6	Persistence/stability of test substance in aquatic solution reported?	1	N	
7	If test material is radiolabelled, were precise position(s) of the labelled atom(s) and the percentage of radioactivity associated with impurities reported?	2	N	
	Method			
8	Reference	1	Y	OECD guidelines for the testing of chemicals No 305B-1996
9	OECD, EU, national or other standard method?	3	Y	OECD
10	Justification of the method/protocol if a nonstandard method was used	2		Not applicable
11	GLP (good laboratory practice)	3	N	
Test organism	***Test organism***			
12	Organism identity: name	n/a	*Y*	Zebra fish, *Brachydanio rerio*
13	Latin or both Latin and common names reported?	1	Y	Both
14	Life cycle age / stage of test organism	1	N	
15	Length and/or weight	1	Y	Mean body length 3.91+/-0.18 cm and mean body weight 0.32+/-0.06 g
16	Sex	1	N	
17	Number of organisms per replicate	1	Y	7
18	Organism loading rate	1	Y	20 mg/L
19	Food type and feeding periods during the acclimation period	1	Y	Fed a commercial fish diet until one day before start of test
	Test design / conditions			
20	Experiment type (laboratory or field)	n/a	Y	Laboratory
21	Exposure pathways (food, water, both)	n/a	Y	Water
22	Exposure duration	n/a	Y	28 days
23	Number of replicates (including controls)	1	Y	
24	Concentrations	1	Y	20 mg/L
25	Food type/composition and feeding periods during the test	1	Y	Fish were fed two hours before water renewal
26	If BCF/BAF derived as a ratio of chemical concentration in the organism and in water, was experiment duration equal to or longer than the time required for the chemical concentrations to reach steady state?	3	Y	28 days

[1] n/a = not applicable

27	If BCF/BAF derived as a ratio of chemical concentration in the organism and in water, were measured concentrations in both water and organism reported?	3	Y	
28	Were concentrations in the test water measured periodically?	1	Y	On three separate days
29	Were the exposure media conditions relevant to the particular chemical reported? (e.g., for metal toxicity - pH, DOC/TOC, water hardness, temperature)	3	Y	Yes, every second day
30	Photoperiod and light intensity	1	Y	12:12
31	Stock and test solution preparation	1	Y	
32	Analytical monitoring intervals	1	Y	Every second day for dissolved oxygen, pH and temperature
33	Statistical methods used	1	Y	
34	Was solubilizer/emulsifier used if the chemical was unstable or poorly soluble?	n/a	N	
	Information relevant to the data quality			
35	Was the test organism relevant to the Canadian environment?	3	Y	
36	Were the test conditions (pH, temperature, DO, etc.) typical for the test organism?	1	Y	
37	Does system type and design (static, semi-static, flow-through; sealed or open; etc.) correspond to the substance's properties and organism's nature/habits?	2	Y	Semi-static
38	Was pH of the test water within the range typical for the Canadian environment (6 to 9)?	1	Y	7.22–7.84
39	Was temperature of the test water within the range typical for the Canadian environment (5 to 27°C)?	1	Y	22–23
40	Was lipid content (or lipid-normalized BAF/BCF) reported?	2	Y	
41	Were measured concentrations of a chemical in the test water below the chemical's water solubility?	3	N	
42	If radiolabelled test substance was used, was BCF determination based on the parent compound (i.e., not on total radiolabelled residues)?	3	N	
	Results			
43	Endpoints (BAF, BCF) and values	n/a	n/a	BCF
44	BAF or BCF determined as: 1) the ratio of chemical concentration in the organism and in water, or 2) the ratio of the chemical uptake and elimination rate constants	n/a	n/a	1
45	Was BAF/BCF was derived from a 1) tissue sample or 2) whole organism?	n/a	n/a	2
46	Was 1) average or 2) maximum BAF/BCF used?	n/a	n/a	1
47	***Score*: %**	***67.9***		
48	***EC reliability code*:**	***2***		
49	***Reliability category (high, satisfactory, low)*:**	***Satisfactory Confidence***		
50	***Comments***	*The present procedure is based on semi-static conditions (renewal of test solutions every 2 days). Therefore, test chemicals with very low water solubility like the disazo dyes can also be characterized as to their bioconcentration potential without adding solvents or other auxiliary substances that may affect the results.*		

Robust Study Summaries Form and Instructions: Aquatic iT				
No.	**Item**	**Weight**	**Yes/No**	**Specify**
1	Reference: Fish toxicity study for CAS# 6250-23-3 (Disperse Yellow 23) Clariant dyestuff product=Foron Yellow E RGFL (Study Submission 2008b)			
2	Substance identity: CAS RN	n/a[1]	Y	6250-23-3
3	Substance identity: chemical name(s)	n/a	Y	Disperse Yellow 23
4	Chemical composition of the substance	2	N	
5	Chemical purity	1	Y	54% DY23
6	Persistence/stability of test substance in aquatic solution reported?	1	N	
Method				
7	Reference	1	N	
8	OECD, EU, national or other standard method?	3	N	
9	Justification of the method/protocol if a nonstandard method was used	2	N	
10	GLP (good laboratory practice)	3		Not applicable (study conducted 1974)
Test organism				
11	Organism identity: name	n/a	Y	Rainbow trout
12	Latin or both Latin and common names reported?	1	N	
13	Life cycle age / stage of test organism	1	Y	
14	Length and/or weight	1	Y	11 cm length, 15 g weight
15	Sex	1	N	
16	Number of organisms per replicate	1	N	
17	Organism loading rate	1	N	
18	Food type and feeding periods during the acclimation period	1	N	
Test design / conditions				
19	Test type (acute or chronic)	n/a	Y	Acute
20	Experiment type (laboratory or field)	n/a	Y	Lab
21	Exposure pathways (food, water, both)	n/a	Y	Water
22	Exposure duration	n/a	Y	48 hours
23	Negative or positive controls (specify)	1	N	
24	Number of replicates (including controls)	1	N	
25	Nominal concentrations reported?	1	N	
26	Measured concentrations reported?	3	N	
27	Food type and feeding periods during the long-term tests	1		Not applicable

[1] n/a = not applicable

28	Were concentrations measured periodically (especially in the chronic test)?	1	N	
29	Were the exposure media conditions relevant to the particular chemical reported? (e.g., for metal toxicity - pH, DOC/TOC, water hardness, temperature)	3	N	
30	Photoperiod and light intensity	1	N	
31	Stock and test solution preparation	1	N	
32	Was solubilizer/emulsifier used if the chemical was poorly soluble or unstable?	1	N	
33	If solubilizer/emulsifier was used, was its concentration reported?	1		
34	If solubilizer/emulsifier was used, was its ecotoxicity reported?	1		
35	Analytical monitoring intervals	1	N	
36	Statistical methods used	1	N	
Information relevant to the data quality				
37	Was the endpoint directly caused by the chemical's toxicity, not by organism's health (e.g., when mortality in the control >10%) or physical effects (e.g., "shading effect")?	n/a	Y	
38	Was the test organism relevant to the Canadian environment?	3	Y	
39	Were the test conditions (pH, temperature, DO, etc.) typical for the test organism?	1	N	Temperature only variable reported (temp is typical for test organism)
40	Does system type and design (static, semi-static, flow-through; sealed or open; etc.) correspond to the substance's properties and organism's nature/habits?	2		Not able to assess
41	Was pH of the test water within the range typical for the Canadian environment (6 to 9)?	1		pH not specified
42	Was temperature of the test water within the range typical for the Canadian environment (5 to 27°C)?	1	Y	20^{o}C
43	Was toxicity value below the chemical's water solubility?	3	N	The reported LC_{50} is 8 orders of magnitude higher than the WS measured in Baughmann and Perenich 1989.
Results				
44	Toxicity values (specify endpoint and value)	n/a	n/a	48-hour LC_{50} >1000 mg/L
45	Other endpoints reported - e.g., BCF/BAF, LOEC/NOEC (specify)?	n/a	N	
46	Other adverse effects (e.g., carcinogenicity, mutagenicity) reported?	n/a	N	
47	***Score*: %**		***17.5***	
48	***EC reliability code*:**		***4***	
49	***Reliability category (high, satisfactory, low)*:**		***Not Satisfactory***	
50	***Comments***			

Robust Study Summaries Form and Instructions: Aquatic iT				
No.	**Item**	**Weight**	**Yes/No**	**Specify**
1	Reference: ETAD: Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers. 2005. Project E 3020: Information received in support of DSL categorization for 12 disperse dyes. (Email correspondence dated October 27, 2005)			
2	Substance identity: CAS RN	n/a[1]	Y	19800-42-1
3	Substance identity: chemical name(s)	n/a	Y	Disperse Orange 29
4	Chemical composition of the substance	2	Y	20% dyestuff, 10% Reax 85A, 70% water
5	Chemical purity	1	Y	Dispersion 20% dyestuff
6	Persistence/stability of test substance in aquatic solution reported?	1	N	
	Method			
7	Reference	1	N	
8	OECD, EU, national or other standard method?	3	Y	OECD 203
9	Justification of the method/protocol if a nonstandard method was used	2		Not applicable
10	GLP (good laboratory practice)	3		Not applicable (study conducted 1990)
	Test organism			
11	Organism identity: name	n/a	Y	zebra fish, *Brachydanio rerio*
12	Latin or both Latin and common names reported?	1	Y	
13	Life cycle age / stage of test organism	1	N	
14	Length and/or weight	1	N	
15	Sex	1		Not applicable
16	Number of organisms per replicate	1	N	
17	Organism loading rate	1	N	
18	Food type and feeding periods during the acclimation period	1	N	
	Test design / conditions			
19	Test type (acute or chronic)	n/a	Y	Acute
20	Experiment type (laboratory or field)	n/a	Y	Lab
21	Exposure pathways (food, water, both)	n/a	Y	Water
22	Exposure duration	n/a	Y	48 hours
23	Negative or positive controls (specify)	1	N	
24	Number of replicates (including controls)	1	N	

25	Nominal concentrations reported?	1	N	

[1] n/a = not applicable

26	Measured concentrations reported?	3	N	
27	Food type and feeding periods during the long-term tests	1		Not applicable
28	Were concentrations measured periodically (especially in the chronic test)?	1	N	
29	Were the exposure media conditions relevant to the particular chemical reported? (e.g., for metal toxicity - pH, DOC/TOC, water hardness, temperature)	3	N	
30	Photoperiod and light intensity	1	N	
31	Stock and test solution preparation	1	N	
32	Was solubilizer/emulsifier used if the chemical was poorly soluble or unstable?	1	N	
33	If solubilizer/emulsifier was used, was its concentration reported?	1		
34	If solubilizer/emulsifier was used, was its ecotoxicity reported?	1		
35	Analytical monitoring intervals	1	N	
36	Statistical methods used	1	N	
Information relevant to the data quality				
37	Was the endpoint directly caused by the chemical's toxicity, not by the organism's health (e.g., when mortality in the control >10%) or physical effects (e.g., "shading effect")?	n/a	Y	
38	Was the test organism relevant to the Canadian environment?	3	N	
39	Were the test conditions (pH, temperature, DO, etc.) typical for the test organism?	1		Not reported
40	Does system type and design (static, semi-static, flow-through; sealed or open; etc.) correspond to the substance's properties and organism's nature/habits?	2		Not able to assess
41	Was pH of the test water within the range typical for the Canadian environment (6 to 9)?	1		pH not specified
42	Was temperature of the test water within the range typical for the Canadian environment (5 to 27°C)?	1		Not specified
43	Was toxicity value below the chemical's water solubility?	3	Y	The reported LC_{50} and WS are within 1 order of magnitude of each other.
Results				
44	Toxicity values (specify endpoint and value)	n/a	n/a	96-hour LC_{50} = 480 mg/L
45	Other endpoints reported - e.g., BCF/BAF, LOEC/NOEC (specify)?	n/a	N	
46	Other adverse effects (e.g., carcinogenicity, mutagenicity) reported?	n/a	N	
47	***Score:. %***			***28.6***
48	***EC reliability code*:**			***4***
49	***Reliability category (high, satisfactory, low)*:**			***Not Satisfactory***
50	***Comments***			

Robust Study Summaries Form and Instructions: Aquatic iT				
No	**Item**	**Weight**	**Yes/No**	**Specify**
1	Reference: ETAD: Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers. 2005. Project E 3020: Information received in support of DSL categorization for 12 disperse dyes. (Email correspondence dated October 27, 2005) (Study Submission 2008a)			
2	Substance identity: CAS RN	n/a[1]	Y	19800-42-1
3	Substance identity: chemical name(s)	n/a	Y	Disperse Orange 29
4	Chemical composition of the substance	2	Y	20% dyestuff, 10% Reax 85A, 70% water
5	Chemical purity	1	Y	Dispersion 20% dyestuff
6	Persistence/stability of test substance in aquatic solution reported?	1	N	
Method				
7	Reference	1	N	
8	OECD, EU, national or other standard method?	3	Y	OECD 201
9	Justification of the method/protocol if a nonstandard method was used	2		Not applicable
10	GLP (good laboratory practice)	3		Not applicable (study conducted 1990)
Test organism				
11	Organism identity: name	n/a	Y	*Scenedesmus subspicatus*
12	Latin or both Latin and common names reported?	1	Y	
13	Life cycle age / stage of test organism	1	N	
14	Length and/or weight	1		Not applicable
15	Sex	1		Not applicable
16	Number of organisms per replicate	1	N	
17	Organism loading rate	1	N	
18	Food type and feeding periods during the acclimation period	1	N	
Test design / conditions				
19	Test type (acute or chronic)	n/a	Y	Acute
20	Experiment type (laboratory or field)	n/a	Y	Lab
21	Exposure pathways (food, water, both)	n/a	Y	Water
22	Exposure duration	n/a	Y	72 hours
23	Negative or positive controls (specify)	1	N	
24	Number of replicates (including controls)	1	N	

[1] n/a = not applicable

25	Nominal concentrations reported?	1	N	
26	Measured concentrations reported?	3	N	
27	Food type and feeding periods during the long-term tests	1		Not applicable
28	Were concentrations measured periodically (especially in the chronic test)?	1	N	
29	Were the exposure media conditions relevant to the particular chemical reported? (e.g., for metal toxicity - pH, DOC/TOC, water hardness, temperature)	3	N	
30	Photoperiod and light intensity	1	N	
31	Stock and test solution preparation	1	N	
32	Was solubilizer/emulsifier used if the chemical was poorly soluble or unstable?	1	N	
33	If solubilizer/emulsifier was used, was its concentration reported?	1		
34	If solubilizer/emulsifier was used, was its ecotoxicity reported?	1		
35	Analytical monitoring intervals	1	N	
36	Statistical methods used	1	N	
Information relevant to the data quality				
37	Was the endpoint directly caused by the chemical's toxicity, not by the organism's health (e.g., when mortality in the control >10%) or physical effects (e.g., "shading effect")?	n/a	Y	
38	Was the test organism relevant to the Canadian environment?	3	Y	
39	Were the test conditions (pH, temperature, DO, etc.) typical for the test organism?	1		Not reported
40	Does system type and design (static, semi-static, flow-through; sealed or open; etc.) correspond to the substance's properties and organism's nature/habits?	2		Not able to assess
41	Was pH of the test water within the range typical for the Canadian environment (6 to 9)?	1		pH not specified
42	Was temperature of the test water within the range typical for the Canadian environment (5 to 27°C)?	1		Not specified
43	Was toxicity value below the chemical's water solubility?	3	Y	EC_{50}s and WS are within 1 order of magnitude of each other.
Results				
44	Toxicity values (specify endpoint and value)	n/a	n/a	72-hr biomass EC_{50} = 6 mg/L
45	Other endpoints reported - e.g., BCF/BAF, LOEC/NOEC (specify)?	n/a	Y	Growth EC_{50} = 86 mg/L; biomass/ growth EC_{10} = 1.7/5.4 mg/L
46	Other adverse effects (e.g., carcinogenicity, mutagenicity) reported?	n/a	N	
47	***Score*: %**		***38.2***	

48	***EC reliability code*:**	***4***
49	***Reliability category (high, satisfactory, low)*:**	***Not Satisfactory***
50	***Comments***	

Robust Study Summaries Form and Instructions: Aquatic iT				
No	**Item**	**Weight**	**Yes/No**	**Specify**
1	Reference: ETAD: Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers. 2005. Project E 3020: Information received in support of DSL categorization for 12 disperse dyes. (Email correspondence dated October 27, 2005) (Study Submission 2008a)			
2	Substance identity: CAS RN	n/a[1]	Y	19800-42-1
3	Substance identity: chemical name(s)	n/a	Y	Disperse Orange 29
4	Chemical composition of the substance	2	Y	20% dyestuff, 10% Reax 85A, 70% water
5	Chemical purity	1	Y	Dispersion 20% dyestuff
6	Persistence/stability of test substance in aquatic solution reported?	1	N	
Method				
7	Reference	1	N	
8	OECD, EU, national or other standard method?	3	Y	OECD 202
9	Justification of the method/protocol if a nonstandard method was used	2		Not applicable
10	GLP (good laboratory practice)	3		Not applicable (study conducted 1990)
Test organism				
11	Organism identity: name	n/a	Y	*Daphnia magna*
12	Latin or both Latin and common names reported?	1	Y	
13	Life cycle age / stage of test organism	1	N	
14	Length and/or weight	1		Not applicable
15	Sex	1		Not applicable
16	Number of organisms per replicate	1	N	
17	Organism loading rate	1	N	
18	Food type and feeding periods during the acclimation period	1	N	
Test design / conditions				
19	Test type (acute or chronic)	n/a	Y	Acute
20	Experiment type (laboratory or field)	n/a	Y	Lab
21	Exposure pathways (food, water, both)	n/a	Y	Water
22	Exposure duration	n/a	Y	24 and 48 hours
23	Negative or positive controls (specify)	1	N	
24	Number of replicates (including controls)	1	N	

[1] n/a = not applicable

25	Nominal concentrations reported?	1	N	
26	Measured concentrations reported?	3	N	
27	Food type and feeding periods during the long-term tests	1		Not applicable
28	Were concentrations measured periodically (especially in the chronic test)?	1	N	
29	Were the exposure media conditions relevant to the particular chemical reported? (e.g., for metal toxicity - pH, DOC/TOC, water hardness, temperature)	3	N	
30	Photoperiod and light intensity	1	N	
31	Stock and test solution preparation	1	N	
32	Was solubilizer/emulsifier used if the chemical was poorly soluble or unstable?	1	N	
33	If solubilizer/emulsifier was used, was its concentration reported?	1		
34	If solubilizer/emulsifier was used, was its ecotoxicity reported?	1		
35	Analytical monitoring intervals	1	N	
36	Statistical methods used	1	N	
Information relevant to the data quality				
37	Was the endpoint directly caused by the chemical's toxicity, not by organism's health (e.g., when mortality in the control >10%) or physical effects (e.g., "shading effect")?	n/a	Y	
38	Was the test organism relevant to the Canadian environment?	3	Y	
39	Were the test conditions (pH, temperature, DO, etc.) typical for the test organism?	1		Not reported
40	Does system type and design (static, semi-static, flow-through; sealed or open; etc.) correspond to the substance's properties and organism's nature/habits?	2		Not able to assess
41	Was pH of the test water within the range typical for the Canadian environment (6 to 9)?	1		pH not specified
42	Was temperature of the test water within the range typical for the Canadian environment (5 to 27°C)?	1		Not specified
43	Was toxicity value below the chemical's water solubility?	3	N	The reported LC_{50} is greater than the WS provided.
Results				
44	Toxicity values (specify endpoint and value)	n/a	n/a	48-hr ELC_{50} = 70 mg/L
45	Other endpoints reported - e.g., BCF/BAF, LOEC/NOEC (specify)?	n/a	Y	24-hr EC_{50} >100 mg/L
46	Other adverse effects (e.g., carcinogenicity, mutagenicity) reported?	n/a	N	
47	***Score*: %**		***29.4***	
48	***EC reliability code*:**		***4***	
49	***Reliability category (high, satisfactory, low)*:**		***Not Satisfactory***	

50	***Comments***	

Robust Study Summaries Form: Aquatic iT				
No	**Item**	**Weight**	**Yes/No**	**Specify**
1	Reference: BASF. 1990. Bericht uber die Prufung der akuten Toxizitit an der Goldorfe (*Leuciscus idus L.,. Goldvariante*). Submitted by ETAD to Environment Canada, August 2008.			
2	Substance identity: CAS RN	n/a[1]		
3	Substance identity: chemical name(s)	n/a		
4	Chemical composition of the substance	2	N	
5	Chemical purity	1	N	
6	Persistence/stability of test substance in aquatic solution reported?	1	N	
Method				
7	Reference	1	N	
8	OECD, EU, national or other standard method?	3	N	
9	Justification of the method/protocol if a nonstandard method was used	2	N	
10	GLP (good laboratory practice)	3		
Test organism				
11	Organism identity: name	n/a	Y	Golden orfe
12	Latin or both Latin and common names reported?	1	Y	
13	Life cycle age / stage of test organism	1	N	
14	Length and/or weight	1	N	
15	Sex	1	N	
16	Number of organisms per replicate	1	N	
17	Organism loading rate	1	N	

[1] n/a = not applicable

18	Food type and feeding periods during the acclimation period	1	N	
Test design / conditions				
19	Test type (acute or chronic)	n/a	Y	Acute
20	Experiment type (laboratory or field)	n/a	N	
21	Exposure pathways (food, water, both)	n/a	N	
22	Exposure duration	n/a	Y	96 hr
23	Negative or positive controls (specify)	1	N	
24	Number of replicates (including controls)	1	N	
25	Nominal concentrations reported?	1	N	
26	Measured concentrations reported?	3	N	
27	Food type and feeding periods during the long-term tests	1		Not applicable
28	Were concentrations measured periodically (especially in the chronic test)?	1	N	
29	Were the exposure media conditions relevant to the particular chemical reported? (e.g., for metal toxicity - pH, DOC/TOC, water hardness, temperature)	3	N	
30	Photoperiod and light intensity	1	N	
31	Stock and test solution preparation	1	N	
32	Was solubilizer/emulsifier used if the chemical was poorly soluble or unstable?	1	N	
33	If solubilizer/emulsifier was used, was its concentration reported?	1	N	
34	If solubilizer/emulsifier was used, was its ecotoxicity reported?	1	N	
35	Analytical monitoring intervals	1	N	
36	Statistical methods used	1	N	
Information relevant to the data quality				
37	Was the endpoint directly caused by the chemical's toxicity, not by the organism’s health (e.g., when mortality in the control >10%) or physical effects (e.g., “shading effect”)?	n/a	N	
38	Was the test organism relevant to the Canadian environment?	3	Y	
39	Were the test conditions (pH, temperature, DO, etc.) typical for the test organism?	1	N	
40	Does system type and design (static, semi-static, flow-through; sealed or open; etc.) correspond to the substance's properties and organism's nature/habits?	2	N	
41	Was pH of the test water within the range typical for the Canadian environment (6 to 9)?	1	N	
42	Was temperature of the test water within the range typical for the Canadian environment (5 to 27°C)?	1	N	

43	Was toxicity value below the chemical's water solubility?	3		
Results				
44	Toxicity values (specify endpoint and value)	n/a		LC_{50} = >100 <220 mg/L
45	Other endpoints reported - e.g., BCF/BAF, LOEC/NOEC (specify)?	n/a		NOEC = 100 mg/L
46	Other adverse effects (e.g., carcinogenicity, mutagenicity) reported?	n/a		
47	***Score*: %**			***9.5***
48	***EC reliability code*:**			***4***
49	***Reliability category (high, satisfactory, low)*:**			***Not Satisfactory***
50	***Comments***			***Not enough data submitted to properly assess the reliability of this study.***

Robust Study Summaries Form: Aquatic iT				
No	**Item**	**Weight**	**Yes/No**	**Specify**
1	Reference: Environment Canada. 1995. NS submission. Submitted to New Substances Branch, Environment Canada under New Substances Notification Program.			
2	Substance identity: CAS RN	n/a	N	
3	Substance identity: chemical name(s)	n/a	Y	
4	Chemical composition of the substance	2	N	
5	Chemical purity	1	N	
6	Persistence/stability of test substance in aquatic solution reported?	1	N	
Method				
7	Reference	1	Y	OECD 203
8	OECD, EU, national or other standard method?	3	Y	
9	Justification of the method/protocol if a nonstandard method was used	2		Not applicable
10	GLP (good laboratory practice)	3	Y	
Test organism				
11	Organism identity: name	n/a	Y	Rainbow trout
12	Latin or both Latin and common names reported?	1	Y	
13	Life cycle age / stage of test organism	1	Y	Mean length 51 mm and mean weight 1.54 g
14	Length and/or weight	1	Y	See above
15	Sex	1		Not applicable
16	Number of organisms per replicate	1	Y	10
17	Organism loading rate	1	Y	

18	Food type and feeding periods during the acclimation period	1	Y	
Test design / conditions				
19	Test type (acute or chronic)	n/a	Y	Acute
20	Experiment type (laboratory or field)	n/a	y	Lab
21	Exposure pathways (food, water, both)	n/a	y	Water
22	Exposure duration	n/a	y	96 hr
23	Negative or positive controls (specify)	1	Y	3
24	Number of replicates (including controls)	1	Y	2
25	Nominal concentrations reported?	1	Y	320–3200 mg/L
26	Measured concentrations reported?	3	N	
27	Food type and feeding periods during the long-term tests	1		Not applicable
28	Were concentrations measured periodically (especially in the chronic test)?	1	N	
29	Were the exposure media conditions relevant to the particular chemical reported? (e.g., for metal toxicity - pH, DOC/TOC, water hardness, temperature)	3	Y	
30	Photoperiod and light intensity	1	Y	
31	Stock and test solution preparation	1	Y	
32	Was solubilizer/emulsifier used if the chemical was poorly soluble or unstable?	1	N	
33	If solubilizer/emulsifier was used, was its concentration reported?	1		
34	If solubilizer/emulsifier was used, was its ecotoxicity reported?	1		
35	Analytical monitoring intervals	1	Y	
36	Statistical methods used	1	Y	
Information relevant to the data quality				
37	Was the endpoint directly caused by the chemical's toxicity, not by the organism’s health (e.g., when mortality in the control >10%) or physical effects (e.g., ”shading effect”)?	n/a	Y	
38	Was the test organism relevant to the Canadian environment?	3	Y	
39	Were the test conditions (pH, temperature, DO, etc.) typical for the test organism?	1	Y	
40	Does system type and design (static, semi-static, flow-through; sealed or open; etc.) correspond to the substance's properties and organism's nature/habits?	2	Y	
41	Was pH of the test water within the range typical for the Canadian environment (6 to 9)?	1	Y	
42	Was temperature of the test water within the range typical for the Canadian environment (5 to 27°C)?	1	Y	

43	Was toxicity value below the chemical's water solubility?	3		Unknown water solubility
Results				
44	Toxicity values (specify endpoint and value)	n/a	n/a	96-hr LC_{50}
45	Other endpoints reported - e.g., BCF/BAF, LOEC/NOEC (specify)?	n/a	N	
46	Other adverse effects (e.g., carcinogenicity, mutagenicity) reported?	n/a	N	
47	***Score*: %**		***77.5***	
48	***EC reliability code*:**		***2***	
49	***Reliability category (high, satisfactory, low)*:**		***Satisfactory Confidence***	
50	***Comments***			

Robust Study Summaries Form and Instructions: Aquatic iT				
No	**Item**	**Weight**	**Yes/No**	**Specify**
1	Reference: Cohle P and R Mihalik. 1991. Early life stage toxicity of C.I. Disperse Blue 79:1 purified presscake to rainbow trout (*Oncorhynchus mykiss*) in a flow-through system.			
2	Substance identity: CAS RN	n/a		
3	Substance identity: chemical name(s)	n/a		Disperse Blue 79:1
4	Chemical composition of the substance	2	Y	
5	Chemical purity	1	Y	96.61
6	Persistence/stability of test substance in aquatic solution reported?	1	N	
Method				
7	Reference	1	Y	
8	OECD, EU, national or other standard method?	3	Y	ASTM 1983
9	Justification of the method/protocol if a nonstandard method was used	2		Not applicable
10	GLP (good laboratory practice)	3	Y	
Test organism				
11	Organism identity: name	n/a		Rainbow trout
12	Latin or both Latin and common names reported?	1	Y	
13	Life cycle age / stage of test organism	1	Y	
14	Length and/or weight	1	Y	
15	Sex	1		Not applicable
16	Number of organisms per replicate	1	Y	20
17	Organism loading rate	1	Y	0.36 to 4.8 µg/L
18	Food type and feeding periods during the acclimation period	1	Y	

Test design / conditions				
19	Test type (acute or chronic)	n/a	Y	Chronic
20	Experiment type (laboratory or field)	n/a	Y	Lab
21	Exposure pathways (food, water, both)	n/a	Y	Water
22	Exposure duration	n/a	Y	122 days
23	Negative or positive controls (specify)	1	Y	Control and carrier blank
24	Number of replicates (including controls)	1	Y	2
25	Nominal concentrations reported?	1	Y	5
26	Measured concentrations reported?	3	Y	
27	Food type and feeding periods during the long-term tests	1	Y	
28	Were concentrations measured periodically (especially in the chronic test)?	1	Y	
29	Were the exposure media conditions relevant to the particular chemical reported? (e.g., for metal toxicity - pH, DOC/TOC, water hardness, temperature)	3	Y	
30	Photoperiod and light intensity	1	Y	
31	Stock and test solution preparation	1	Y	
32	Was solubilizer/emulsifier used if the chemical was poorly soluble or unstable?	1	Y	
33	If solubilizer/emulsifier was used, was its concentration reported?	1	Y	
34	If solubilizer/emulsifier was used, was its ecotoxicity reported?	1	Y	No tox value but it was used as a control
35	Analytical monitoring intervals	1	Y	
36	Statistical methods used	1	Y	
Information relevant to the data quality				
37	Was the endpoint directly caused by the chemical's toxicity, not by the organism's health (e.g., when mortality in the control >10%) or physical effects (e.g., "shading effect")?	n/a	Y	
38	Was the test organism relevant to the Canadian environment?	3	Y	
39	Were the test conditions (pH, temperature, DO, etc.) typical for the test organism?	1	Y	
40	Does system type and design (static, semi-static, flow-through; sealed or open; etc.) correspond to the substance's properties and organism's nature/habits?	2	Y	Flow-through
41	Was pH of the test water within the range typical for the Canadian environment (6 to 9)?	1	Y	
42	Was temperature of the test water within the range typical for the Canadian environment (5 to 27°C)?	1	Y	

43	Was toxicity value below the chemical's water solubility?	3		Cannot assess. Highest dose tested believed to be at limit of solubility.
Results				
44	Toxicity values (specify endpoint and value)	n/a	n/a	NOEC >0.005 mg/L
45	Other endpoints reported - e.g., BCF/BAF, LOEC/NOEC (specify)?	n/a		
46	Other adverse effects (e.g., carcinogenicity, mutagenicity) reported?	n/a		
47	***Score*: %**	***97.7***		
48	***EC reliability code*:**	***1***		
49	***Reliability category (high, satisfactory, low)*:**	***High Confidence***		
50	***Comments***			

Appendix 2 – PBT Model Inputs Summary Table

Solvent Red 23 (85-86-9)

	Phys-Chem/Fate	**PBT Profiling**	**Ecotoxicity**
Model Input Parameters	EPIWIN Suite (all models, including: AOPWIN, KOCWIN, BCFWIN BIOWIN and ECOSAR)	Canadian-POPs (including: Catabol, BCF Mitigating Factors Model, OASIS Toxicity Model)	Artificial Intelligence Expert System (AIES)/ TOPKAT/ ASTER
SMILES Code[1]	x	x	x

[1] The SMILES Code for Solvent Red 23 was used to generate the model results.

Appendix 3. Estimated exposures of general population to Solvent Red 23 in cosmetics and personal care products based on ConsExpo version 4.1 (ConsExpo 2006; RIVM 2006)

Cosmetic and personal care product cenario	Assumptions	**Estimated exposure**
Lip-gloss/lip-balm[1]	Weight percent of Solvent Red 23: 1% (CNS 2009) Oral route: Exposure type: direct intake Amount product ingested: 0.01 g - assumed entire product is taken in orally (RIVM 2006) Frequency of 1460 x/year (RIVM 2006)	**Oral** **Acute internal dose 0.001 mg/kg** **Chronic internal dose 0.006 mg/kg bw per day**
Bath preparation - liquid soap	Weight percent of Solvent Red 23: 0.1% (CNS 2009) Dermal route: Exposure type: instant application Exposed area: Body area – head area of 16900 cm2 (Health Canada 1995), Amount product applied: 8.7 g (26.1g w/dilution factor 3) frequency of 329×/year (RIVM 2006) uptake fraction: 0.26 (Collier et al. 1993)	**Dermal** **Acute internal dose 0.03 mg/kg** **Chronic internal dose 0.03 mg/kg bw per day**
Massage oil	Weight percent of Solvent Red 23: 0.1% (CNS 2009) Dermal route: Exposure type: instant application Exposed area: Body area – head area of 16900 cm2 (Health Canada 1995), Amount product applied: 8 g frequency of 24×/year (RIVM 2006) uptake fraction: 0.26 (Collier et al.1993)	**Dermal** **Acute internal dose 0.03 mg/kg** **Chronic internal dose 0.002 mg/kg bw per day**
Hair conditioner	Weight percent of Solvent Red 23: 0.1% (CNS 2009) Dermal route: Exposure type: instant application Exposed area: hands area+ ½ of head area, of 1540 cm2 (Health Canada 1995), Amount product applied: 14 g (54 g w/dilution factor 3.9) frequency of 104×/year (RIVM 2006) uptake fraction: 0.26 (Collier et al.1993)	**Dermal** **Acute internal dose 0.05 mg/kg** **Chronic internal dose 0.015 mg/kg-bw per day**
Hair dye	Weight percent of Solvent Red 23: 1% (CNS 2009) Dermal route: Exposure type: instant application Exposed area: ½ of head area, of 638 cm2 (Health Canada 1995)	**Dermal** **Acute internal dose 3.67 mg/kg** **Chronic internal**

	Amount product applied amount: 100 g, Frequency of 10/year (RIVM 2006) Uptake fraction: 0.26 (Collier et al. 1993)	**dose** **0.10 mg/kg-bw per day**
Body lotion	Weight percent of Solvent Red 23: 0.3% (CNS 2009) Dermal route: Exposure type: instant application Exposed area: Body area – head area of 16900 cm2 (Health Canada 1995), Amount product applied: 8 g frequency of 730×/year (RIVM 2006) uptake fraction: 0.26 (Collier et al. 1993)	**Dermal** **Acute internal dose** **0.09 mg/kg** **Chronic internal dose** **0.18 mg/kg-bw per day**
Facial cleanser	Weight percent of Solvent Red 23: 1% (CNS 2009) Dermal route: Exposure type: instant application Exposed area: ½ of head area, of 638 cm2 (Health Canada 1995), Amount product applied amount: 0.25g (25 g with 10% retention factor) Frequency of 730/year (RIVM 2006) Uptake fraction: 0.26 (Collier et al. 1993)	**Dermal** **Acute internal dose** **0.009 mg/kg** **Chronic internal dose** **0.018 mg/kg-bw per day**
Nail polish	Weight percent of Solvent Red 23: 10% (CNS 2009) Dermal route: Exposure type: instant application Exposed area: 4 cm^2 (Health Canada 1995), Amount product applied amount: 0.05g Frequency of 156x/year (RIVM 2006) Uptake fraction: 0.26 (Collier et al. 1993)	**Dermal** **Acute internal dose** **0.02 mg/kg** **Chronic internal dose** **0.008 mg/kg-bw per day**

[1] ConsExpo scenario for lipstick is used in this instance. Chronic internal dose for children age 5-11 with body weight of 31 kg is estimated to be 0.01 mg/kg bw per day.

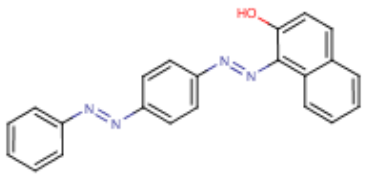
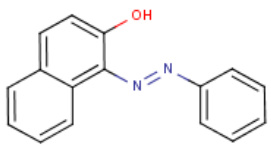
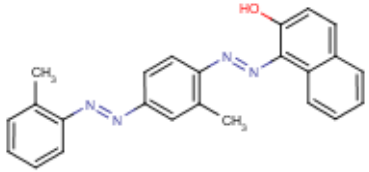
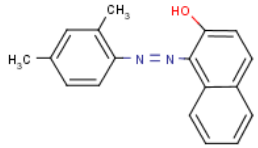
Appendix 4: Summary of health effects information for Solvent Red 23 (*CASRN: 85-86-9*)

Endpoint	Lowest effect levels[1]/Results
Laboratory animals and *in vitro*	
Acute toxicity	**Lowest oral LD_{50}[1] (Rat)** >16000 mg/kg bw. SCCP concluded that solvent red 23 has low oral toxicity following single exposure (SCCP, 2005).
Short-term repeated-dose toxicity	No adequate studies were identified. A 21 day dermal toxicity study was performed on rabbits. The method was not described, and was the study was considered inadequate by the SCCP (SCCP, 2005).
Subchronic toxicity	No adequate studies were identified. A 90 day dermal toxicity study was performed on rabbits. The method was not described, and was the study was considered inadequate by the SCCP (SCCP, 2005).
Chronic toxicity/ carcinogenicity	**Oral:** 83 male and 54 female mice were exposed to a 1 % in oil solution to 2 mg/day (regardless of weight) in feed. Lung tumours were evident but were not considered to be increased over controls (SCCP 2005). Wistar rats (5/sex) were fed 40000 mg/kg of diet for 18 months. No tumours were evident. No information on survival was reported (SCCP 2005). Sixteen female rats were exposed by gastric instillation to a 1 ml, 0.1 % (w/v) solution in sesame oil, 5x/wk for 25 weeks. No excess tumours were observed (SCCP 2005). **Dermal:** Swiss Webster mice (50/sex) were exposed by skin painting. 0.1 ml of a 1 % solution was applied to depilated skin (6 cm^2) (approx. 40 mg/kg bw/day) once weekly for 18 months. No increased tumour incidence was observed. No changes in body weight or survival were noted (SCCP 2005). **Subcutaneous Injection:** Two groups of 10 female mice were dosed repeatedly (number of injections not specified) with 0.25 ml of a saturated solution of Solvent Red 23 or 5 mg of Solvent red 23 crystals. No tumours were reported (SCCP 2005). **Intramuscular Injection:** Long-Evans rats (8, age 27 d) were injected with 0.5 ml of a 0.5% (w/v) solution in sesame oil in the thigh muscle. No tumours were observed at necropsy after 40 weeks (SCCP 2005). **NOTE:** None of theses studies were considered adequate, by IARC, to classify Solvent red 23 as to its carcinogenicity.
Developmental toxicity	Solvent red 23 was administered to pregnant mice orally on days 8-12 of gestation. Male offspring were necropsied at 48-50 days of age and examined for abnormalities in testicular development. No treatement-related effects were observed. (Gray & Ostby 1993).
Reproductive toxicity	No studies were identified that investigated the potential toxicity of Solvent red 23 on development.
Genotoxicity and related endpoints: *in vivo*	**Comet Assay** **Positive:** Male ddY mice (4/group) were administered 2000 mg/kg orally. Mice were sacrificed at 0, 8, 3 or 24 hours. Various tissues were examined, by

Endpoint	Lowest effect levels[1]/Results
	comet assay, for DNA breaks. Increased DNA migration (indicative of DNA damage) was observed in the stomach, colon, and bladder of mice sacrificed at 3 hrs, but not in mice of the 0, 8, or 24 hour groups (Tsuda et al. 2000).
Genotoxicity and related endpoints: *in vitro*	**Mutagenicity** **Negative:** Ames assay carried out with purified test compound in *Salmonella typhimurium* tester strains TA98 and TA100 in the presence of S9 mix. Protocol may not have been appropriate for the class of compound. (Miyagoshi et al. 1985) **Positive:** Ames assay carried out with commercial dye preparations in *Salmonella typhimurium* tester strains TA98 and TA100 in the presence of S9 mix. Authors ascribed activity to contamination with materials from synthesis (4-aminoazobenzene) (Miyagoshi et al. 1985). **Negative:** No increased cell killing (indicator of genotoxicity) was observed in the recombinationless *E. coli* WP2/WP67 or *B. subtilis* 45T rec-assays (Kada et al. 1972; Mamber et al. 1983). **Chromosomal Aberrations** **Positive:** Chinese hamster ovary (CHO) cells were treated with solvent red 23 without activation. An increased number of breaks per metaphase were observed (Au & Hsu 1979). **Negative:** Human lymphocytes exposed *in vitro* to doses up to 100 µg/ml did not have any increase in chromosomal aberrations (SCC 1988).
Irritation/sensitization	Rabbits exposed dermally to Solvent Red 23 showed only slight signs of irritation. FDA classified as slightly irritating. SCC concluded that Solvent Red 23 has maginal irritant properties (SCCP 2005). Rabbits were exposed to Solvent Red 23 in their eyes. The substance was evaluated as slightly irritating. SCC concluded that Solvent Red 23 has marginal irritant properties (SCCP 2005). Groups of 6 animals exposed to either 0.1 % (intradermally) or 1.0% (topically) did not react to challenge doses of 0.001, 0.01, 0.1% Solvent Red 23. However, initial treatment with several other substances (p-phenylenediamine, p-aminoazobenzene) did cause cross-sensitization to Solvent Red 23 (SCCP 2005).

LC_{50}, median lethal concentration; LD_{50}, median lethal dose; LOEC, lowest-observed-effect concentration; LOEL, lowest-observed-effect level; NOEL, no-observed-effect level; NOEC, no-observed-effect concentration.

Appendix 5: Summary of available information on carcinogenicity and genotoxicity for Solvent Red 23 and substances considered to be analogues to Solvent Red 23 in this assessment

Substance name	Solvent Red 23	Sudan I	Sudan IV	Sudan II
Substance Identity				
CAS RN	85-86-9	842-07-9	85-83-6	3118-97-6
Other Identifiers	Sudan III, CI 26100	Solvent Yellow 14, CI 12055	Solvent Red 24, CI 26105	C.I. Solvent Orange 7
Formula	$C_{22}H_{16}N_4O$	$C_{16}H_{12}N_2O$	$C_{24}H_{20}N_4O$	$C_{18}H_{16}N_2O$
M.W.	352.39	248.28	380.45	276.3
Water solubility	2.60E-03 mg/L (estimated)	0.67 mg/L (estimated)	2.03E-04 mg/L (estimated)	0.055 mg/L (estimated)
Log Kow	7.63 (estimated)	5.51 (estimated)	8.72 (estimated)	6.600 (estimated)
Structure				
International Classifications	• IARC Group 3 • Predicted cleavage to aromatic amine listed on EU22 (i.e. 4-aminoazobenzene, CAS RN 60-09-3)	• Carc. 2 (CLP - Europe) • Mut. 2 (CLP - Europe) • IARC Group 3	• IARC Group 3 • Predicted cleavage to aromatic amines listed on EU22 (i.e. o-aminoazotoluene CAS RN 97-56-3, and 2-toluidine CAS RN 95-53-4)	• IARC Group 3
Carcinogenicity and Genotoxicity				
Carcinogenicity	**Oral** • 83 male and 54 female mice were exposed to a 1 % in oil solution to 2 mg/day (regardless of weight) in feed. Lung tumours were evident but were not considered to be increased	**Oral** • F344/N rats (50/sex/group) were fed 250 or 500 ppm in the diet for 103 weeks. Carcinogenic in male and female rats based on increased incidence of neoplastic liver nodules (NTP	**Oral** • Heterozygous type B mice were administered 2 mg/animal/day as a 1% solution in grapeseed or beachnut oil. Sacrificed was at 500-700 days. Lymphomas and/or lung tumours	**Oral** • Albino mice (15/sex) were fed diets containing 1000 mg Sudan II per kg of diet for 52 weeks. 11 males and 10 females survived longer than 20 weeks and the experiment was terminated at 90 weeks. 3

Substance name	Solvent Red 23	Sudan I	Sudan IV	Sudan II
Carcinogenicity	over controls (IARC 1975). • Wistar rats (5/sex) were fed 40000 mg/kg of diet for 18 months. No tumours were evident. No information on survival was reported (IARC 1975). • Sixteen female rats were exposed by gastric instillation to a 1 ml, 0.1 % (w/v) solution in sesame oil, 5x/wk for 25 weeks. No excess tumours were observed (IARC 1975). **Dermal** • Swiss Webster mice (50/sex) were exposed by skin painting. 0.1 ml of a 1 % solution was applied to depilated skin (6 cm^2) (approx. 40 mg/kg bw/day) once weekly for 18 months. No increased tumour incidence was observed. No changes in body weight or survival were noted (IARC 1975). • All studies reported here were considered to be inadequate for evaluation by IARC (1975).	2010). • $B6C3F_1$ mice (50/sex/group) were fed 500 or 1000 ppm in the diet for 103 weeks. No evidence of carcinogenicity was observed in either sex (NTP 2010). * Other outdated or inadequate studies cited in IARC 1975.	developed in 2/81 males and 7/25 females vs. 7/109 and 11/59 in male and female controls, respectively. One multiple adenoma developed in a treated female (IARC 1975). • 20 rats were fed 1000 mg/kg of diet for life. 3 rats survived > 1 year. Two hepatomas were observed. No control data was reported (IARC 1975). **Dermal** • Mice (161 total) were painted 3x/week for 43 weeks with 2.5% in olive oil while receiving a total of 7 s.c. injections of test substance over the same period. No tumours were observed in the 64 mice surviving to 312 days. Hyperplasia of the epithelial layer was observed in 18 animals (IARC 1975). • All studies reported here were considered inadequate for evaluation by IARC (1975)	males and 1 female developed benign intestinal tumours vs. 1 animal in the control group IARC 1975). • Rats (number not specified) were exposed to 1000 or 2500 mg/kg of diet for 2 years. Increased death was observed at both exposure levels. No tumours were observed (IARC 1975). • Rats (20/sex/group) were fed 300, 7500, or 15000 mg per kg of diet. 13/40 animals survived at the low dose. All animals of the middle and high dose groups died within 40 and 20 weeks, respectively (IARC 1975) **Implantation** • 60 mice received paraffin wax pellets weighing 15 – 17 mg, that were composed of 12.5% Sudan II. Pellets were implanted into the urinary bladder. After 40 weeks the study was terminated and bladder carcinomas were observed to have occurred in 43/44 surviving animals. 6/142 control animals, receiving paraffin wax alone, developed bladder carcinomas (IARC 1975). • All studies reported here were considered inadequate for evaluation by IARC (1975)

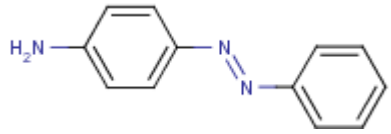
Substance name		Solvent Red 23	Sudan I	Sudan IV	Sudan II
Genotoxicity/ Mutagenicity	*in vivo*	**DNA Damage** • **Comet Assay:** Positive in Male ddY mice (4/group) were administered 2000 mg/kg orally. Mice were sacrificed at 0, 8, 3 or 24 hours. Various tissues were examined, by comet assay, for DNA breaks. Increased DNA migration (indicative of DNA damage) was observed in the stomach, colon, and bladder of mice sacrificed at 3 hrs, but not in mice of the 0, 8, or 24 hour groups (Tsuda et al. 2000).	**Sister Chromatid Exchange** • Positive in mice (NTP 2010). **Chromosomal Aberration** • Positive in mice (NTP 2010). **Micronucleus Assay** • Equivocal evidence in male $B6C3F_1$ mice (NTP 2010). **Reciprocal Translocation** • Negative in *Drosophila melanogaster* (NTP 2010). **Sex-linked Recessive Lethal Mutation** • Negative in *Drosophila melanogaster* (NTP 2010).	No Data	No Data
Genotoxicity/ Mutagenicity	*in vitro*	**Bacterial Mutagenicity** • **Negative:** Ames assay carried out with purified test	**Bacterial Mutagenicity** • **Positive:** In *Salmonella typhimurium* (strain not	**Bacterial Mutagenicity** • **Positive:** Ames assay, preincubation using Prival	**Bacterial Mutagenicity** • **Positive:** Ames assay with metabolic activation (Garner

Substance name		Solvent Red 23	Sudan I	Sudan IV	Sudan II
		compound in *Salmonella typhimurium* tester strains TA98 and TA100 in the presence of S9 mix. No Prival method used. (Miyagoshi et al. 1985). • **Positive:** Ames assay carried out with commercial dye preparations in *Salmonella typhimurium* tester strains TA98 and TA100 in the presence of S9 mix. Authors ascribed activity to contamination with materials from synthesis (4-aminoazobenzene). No Prival method used (Miyagoshi et al. 1985). • **Negative:** No increased cell killing (indicator of genotoxicity) was observed in the recombinationless *E. coli* WP2/WP67 or *B. subtilis* 45T rec-assays. No Prival method used (Kada et al. 1972; Mamber et al. 1983). **Chromosomal Aberrations** • **Positive:** Chinese hamster ovary (CHO) cells were treated with solvent red 23 without activation. An increased number of breaks per metaphase were observed (Au & Hsu 1979). • **Negative:** Human lymphocytes exposed *in vitro* with up to 100 µg/mL did not have any increase in	stated) (NTP 2010). **Mammalian Cell Mutagenicity** • **Positive:** Mouse lymphoma (L5178Y tk^+/tk^-) assay (NTP 2010). **Sister Chromatid Exchange** • **Positive:** In Chinese hamster ovary cells (CHO) (NTP 2010). **Chromosomal Aberration** • **Negative:** In Chinese hamster ovary cells (CHO) (NTP 2010).	method, strain TA98 (Zhou et al. 1987). • **Negative:** Using purified sample **i**n TA98 and TA100 with and without activation (standard method). Positive in TA98 and TA100 using commercial sample with standard activation protocol (Miyagoshi et al. 1985) • **Inconclusive:** testing in *Salmonella typhimurium* (Ames assay) (Brown et al. 1978). **Cell Transformation** • **Positive:** In Syrian hamster embryo (SA7/SHE) cells with viral enhancement (Heidelberger et al. 1983).	and Nutman 1977; Kier et al. 1986). • **Equivocal:** Rec-assay in *Bacillus subtillus* (H17 vs. M45T) (Liefer et al 1981). **Mammalian Cell Mutagenicity** • **Negative:** Mouse lymphoma (L5178Y (tk+/tk-) assay with and without activation (Seifried et al 2006).

Substance name		Solvent Red 23	Sudan I	Sudan IV	Sudan II
		chromosomal aberrations (SCC 1988).			

Appendix 6: Summary of available information on carcinogenicity and genotoxicity for potential products of azo reductive cleavage of Solvent Red 23[19,20]

4-aminoazobenze



CAS RN	60-09-3
Formula	$C_{12}H_{11}N_3$
M.W.	197.24
Classifications	• IARC Group 2B • Carc. Cat 2 (EU)

Carcinogenicity

Oral

16 male rats were fed approximately 100-500 mg/kg bw/day in a low protein diet (to promote liver tumour formation) until death. Seven liver tumours were observed, 2 were malignant. 8 male and 8 female rats fed the same diet without 4-aminoazobenzene had no liver abnormalities (BIBRA 1989).

8 male and 7 female rats fed 75 mg/kg bw/day in a low protein diet did not induce any liver tumours (BIBRA 1989).

44 male rats were fed 40 – 90 mg/kg bw /day in standard diets for 40-60 wks. No evidence of carcinogenicity was observed (BIBRA 1989).

30 Rats fed approximately 35 mg/kg bw/day in the diet for 3-9 wks showed no increased tumour incidence when observed 18 months later regardless of treatment with or without an enhancer of tumour formation (BIBRA 1989).

Thirty female mice were fed 52 mg/kg bw/day in feed for 10 months. Observations were made for an additional 7 months. Malignant tumours were observed in 22 of the 30 mice. Mice fed 27 mg/kg bw/day in the same study showed no evidence of carcinogenicity (BIBRA 1989).

Dermal

Six male rats were exposed, by skin painting, 2 times per week until death. Exposure was to approximately 5-20 mg/kg bw. All six rats developed benign and/or malignant skin tumours after 97 weeks of exposure (BIBRA 1989).

Subcutaneous Injection

Thirty-six mice were fed normal or low-portein diets and injected SC with about 250 mg/kg bw once every 2 weeks for life. No increased incidence of carcinogenicity was observed (BIBRA 1989).

Mice (number and sex not provided) receiving 4 injection of approx. 200 mg/kg bw before weaning were observed until 1 year of age. Liver tumours were observed as well as an increased incidence of malignant tumours of the lymphatic system (BIBRA 1989).

Intraperitoneal Injection

20 female rats were administered 7.5-10 mg/kg bw 3 times per week for 5 weeks. No evidence of carcinogenicity was present after 1 year of observation (BIBRA 1989)

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[19] Health effects data for the potential products of azo reductive cleavage were gathered predominantly from secondary sources, which are referenced here.

[20] 1-((4-aminophenyl)azo)-2-naphthalenol is also a potential product of azo reductive cleavage of Solvent Red 23. This product has no associated CAS RN and no available health effects data and it has therefore not been presented in this table.

242 male mice received one or four injections of approximately 3 mg/kg bw or more before weaning. Examination at 9-12 months of age detected malignant liver tumours in 190 of the 242 mice (BIBRA 1989).

Genotoxicity

In vivo

Chromosome damage
Positive: Rats treated with single doses (36 or 143 mg/kg), intraperitoneally, had significant increases in the number of double strand breaks in liver DNA isolated at 4, 12, or 24 h after exposure (BIBRA 1989).
Positive: Increased DNA fragmentation was detected, by the alkaline elution assay, in the liver DNA of rats exposed to 1.42 mg/kg (BIBRA 1989).

Sister Chromatid Exchange
Positive: Swiss male mice were exposed intraperitoneally to 0, 31, or 62 mg/kg**.** A dose related increase in SCE was observed in the bone marrow cells of the mice (BIBRA 1989).

In vitro

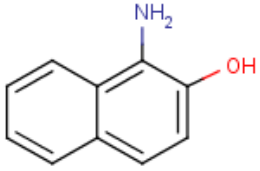
Bacterial Mutagenicity
Positive (bacterial reverse mutation): Ames test in various strains of *Salmonella typhimurium* with metabolic activation, but not without (reviewed in BIBRA 1989).
Positive (host-mediated assay): Mice received 125 mg/kg by intramuscular injection followed by Intraperitoneal injections of bacteria. Increased reverse mutation was seen in one strain of *S. typhimurium* (BIBRA 1989).
Negative (host-mediated assay): Mice exposed orally to 500 mg/kg followed by IP injection of a second strains of *S. tyhphimurium* (not specified in secondary reference).
Negative (bacterial reverse mutation): Ames test using *E. coli* tester strains with or without metabolic activation (BIBRA 1989).

Mammalian Cell Mutagenicity
Positive (Mouse L5178Y/TK assay): The rate of forward mutation was increased when mouse lymphoma L5178Y/TK cells were exposed to the test substance with metabolic activation (BIBRA 1989).

Unscheduled DNA Synthesis
Positive: Incubation of freshly isolated rat hepatocytes with the test substance resulted in a 124% increase in UDS as compared to controls (BIBRA 1989).

Cell Transformation
Positive: Exposure induced transformation in various types of mammalian cells. However, it did not induce transformation in human lung cells with or without metabolic activation (BIBRA 1989).

1-amino-2-napthol



CAS RN	2834-92-6 1198-27-2 (Cl^- salt)
Formula	$C_{10}H_9NO$
M.W.	159.187
Classifications	N/A

Carcinogenicity

Implantation: Paraffin pellets containing 1-amino-2-napthol into the bladders of mice increased the incidence of squamous metaplasias. This study is not considered strong evidence of carcinogenicity because paraffin pellets themselves can induce the proliferation of epithelial cells in the urinary bladder (BfG 2003).

Genotoxicity

In vivo

No Data.

In vitro

Bacterial Mutagenicity
Positive (bacterial reverse mutation): An increased incidence of reverse mutation was observed in *S. tyhphimurium* tester strains TA100 and TA98 when 1-amino-2-napthol was subjected to caecal reduction before incubation with bacteria (Dillon et al. 1994).

p-phenylenediamine

CAS RN	106-50-3
Formula	$C_6H_8N_2$
M.W.	108.143
Classifications	N/A

Carcinogenicity

Dermal
Groups of 50 female Swiss mice, 7 weeks of age, received 5 or 10% in acetone 2x weekly applied to shaved interscapular skin. Exposure continued until death or euthanasia when moribund. No increased incidence of was observed (SCCP 2006).

Groups of 5 female rabbits were exposed to 5 or 10% in acetone 2x weekly for 85 weeks. No increased tumour incidence was observed (SCCP 2006).

Genotoxicity

In vivo

Micronucleus assay
Negative: Rats exposed by gavage to 300 mg/kg twice with a 24 hrs in between doses did not have increased incidence of micronucleated polychromatic erythrocytes (Hossack and Richardson 1977). Negative results were obtained when CD-1 mice where exposed to 25, 50, or 100 mg/kg bw by ip injection (SCCP 2006). Negative in Wistar rats exposed orally to 25, 50, or 100 mg/kg bw (SCCP 2006).

Unscheduled DNA Synthesis
Negative: Wistar rats exposed to 50 or 100 mg/kg bw orally did not have an increased incidence of UDS (SCCP 2006).

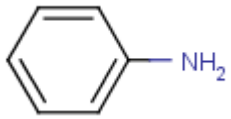
In vitro

Bacterial Mutagenicity
Negative (bacterial reverse mutation): No increase in mutation frequency was observed in TA98, TA100, TA102 or TA1538 *S. typhimurium* strains with activation (SCCP 2006).
Positive (bacterial reverse mutation): Increased mutagenicity (100-fold) in *Salmonella typhimurium* TA98 and TA98NR in the presence of rat liver S9 (SCCP 2006).

Mammalian Mutagenicity
Positive (Mouse L5178Y/TK assay): L5178Y mouse lymphoma cells exposed with and without activation. Increase in mutagenicity was dose-related (SCCP 2006).
Negative (Mouse L5178Y/*hprt*): L5178Y mouse lymphoma cells exposed with and without activation. No increased mutation frequency was observed (SCCP 2006).

Chromosome Aberrations
Positive: CHO-K1 cells exposed to 1,4'-diaminobenzene had a slight increase in the percentage of aberrant cells compared to controls (SCCP 2006).

Aniline



CAS RN	62-53-3
Formula	C_6H_7N
M.W.	93.1283
Classifications	• IARC Group 3 • B2; (probable human carcinogen) (US EPA) • Car. Cat 3 (EU • Muta. Cat. 3 (EU)

Carcinogenicity

Oral
F344 rats (130 per sex/group) were fed aniline hydrochloride at doses of 0, 10, 30 or 100 mg/kg-bw/day for 104 weeks. There was an increased incidence of primary splenic sarcomas at the high dose (EU 2004).

F344 rats (50/sex/group) were fed a diet containing 0.3 or 0.6% aniline hydrochloride (174.4 or 360.5 mg/kg bw/d) for 103 weeks. A dose-related increase in tumours of the spleen was observed. There were also increased incidences of mesenchymal tumours (hemangiosarcoma, fibrosarcoma, fibroma, and sarcoma) (EU 2004).

B6C3F1 mice (50/group) were administered feed containing aniline hydrochloride at concentrations of 0, 0.6, and 1.2% (0, 737 or 1510 mg/kg bw/d in males, 0, 733, or 1560 mg/kg bw/d in females) for 103 weeks followed by 4 weeks of observation. No tumours were considered to be related to test-substance exposure (EU 2004).

Genotoxicity

In vivo

Micronucleus assay
Equivocal: Multiple micronucleus assays were carried out by different groups. Four assays in mice gave positive results at the highest dose tested, while one positive and one negative were observed in rat (EU 2004).

Chromosomal Aberration
Negative: No increase in chromosomal aberrations was observed in mouse bone marrow cells in mice given two doses of 220, 300, or 380 mg/kg intraperitoneally 24 hrs apart. Sampling was performed at 16, 20, and 24 hours after the second administration. Clinical symptoms were observed (EU 2004).

Sister Chromatid Exchange
Positive: A weak dose-dependent increase was observed the bone marrow cells of groups of 3 – 9 mice exposed to doses in the range of 61 to 420 mg/kg intraperitoneally (EU 2004).

DNA Strand Breaks
Positive: Rats and mice were exposed to aniline via intraperitoneal administration. Breaks were increased in rat livers and kidneys but negative in rat spleen. Negative results were obtained for mouse liver, kidney, and bone marrow (EU 2004).

Dominant-Lethal Assay
Inconclusive: Groups of 40 males were exposed to 47, 150 or 200 mg/kg bw/day intraperitoneally each day for 5 days. At the high dose, number of live implants was decreased slightly, but was statistically significant. The number of early deaths was also increased. However, the findings were confined to a group of 7 of 40 mice with clearly reduced numbers of live implants and an elevated number of early dead implants. These results were considered inconclusive by the EU (EU 2004).

In vitro

Bacterial Mutagenicity
Negative: in multiple studies using *Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537 with and without metabolic activation (EU 2004).
Positive (host-mediated assay): 10 rats received 300 mg/kg orally. Urine was collected 24 hrs later and extracted with ether. The extracts were tested using *Salmonella typhimurium* strains TA98 and TA100. A clear dose-dependent response was observed in TA98 with metabolic activation (EU 2004).

Mammalian Cell Mutagenicity
Equivocal: Five mouse lymphoma assays were carried out by five different groups. One clear positive result was obtained with and without metabolic activation. Three weak positives were reported, all at the highest exposure. One negative result was obtained in a study that only tested in the presence of a metabolic activation system (EU 2004).

Positive: Increased mutation frequencies were observed in an HPRT assay using V79 cells when metabolic activation was used. The test was negative without activation. Postives were only observed at extremely high doses. This study was poorly documented (EU 2004).

Chromosomal Aberration
Positive: In CHO cells, high doses of aniline (1600 and 5000 µg/mL) caused increased chromosomal aberrations when tested in the presence of metabolic activation. Doses in the same range also increased chromosomal aberrations in CHL and V79 cells (EU 2004).

Sister Chromatid Exchange
Positive: SCE was induced in human fibroblasts lacking significant cytochrome p450 and NADPH-cytochrome p450 reductase. SCE was also induced in concanavalin A induced T lymphocytes from whole blood cultures; however, the effect was not seen in cultures with purified lymphocytes whereas a marginal increase of SCE was observed when purified lymphocytes were exposed to aniline in the presence of 1000 µg/ml hemoglobin. The EU noted that this study provides evidence that erythrocytes contribute to the transformation of aniline into genotoxic intermediates (EU 2004).

Unscheduled DNA Synthesis
Negative: No increase in DNA repair was observed in primary human hepatocytes or in rat hepatocytes (EU 2004).

DNA Strand Breaks
Equivocal: A very high dose (21.5 mM) caused an increase in DNA strand breaks in mouse lymphoma cells with metabolic activation. The authors interpreted this result as equivocal. No increase was observed without metabolic activation (EU 2004).

Appendix 7: Summary of (Q)SAR Results for Solvent Red 23 and Predicted Metabolites

Carcinogenicity

CAS RN	DEREK (2008)	CASETOX (2008)					TOPKAT (2008)			
	Cancer	m-rat	f-rat	m-mice	f-mice	NTP Rodent	NTP m-rat	NTP f-rat	NTP m-mouse	NTP f-mouse
85-86-9 (parent)	P	IC	IC	N	N	IC	P	ND	P	IC
60-09-3	P	IC	IC	N	N	P	P	IC	N	N
62-53-3	ND	P	P	N	N	P	P*	P*	N*	N*
106-50-3	P	N	N	N	N	N	N*	N*	P	N*
2834-92-6	ND	N	N	N	N	P	P	N	N	N

Genotoxicity

CAS RN	Ames			ChrAb		Micronuclei Induction	Mouse Lymphoma mutation
	Derek	CT	TK	Derek	CT#	CT	CT
85-86-9 (parent)	P	N	IC	ND	IC	N	IC
60-09-3	P	N	P*	ND	P	IC	P
62-53-3	ND	N	N*	ND	P	P	P
106-50-3	P	N	P*	P	P	N	P
2834-92-6	ND	N	P	ND	N	N	N

CAS RN – Chemical Abstracts Registry Number
m – male
f – female
NTP –
P – positive
N – negative
IC – inconclusive
ND – not in domain of model
ChrAb – chromosomal aberration
CT – CASETOX
TK – TOPKAT
– *in vitro* test (in cultured CHO cells)