Screening Assessment for the Challenge

Benzoic acid, 2,3,4,5-tetrachloro-6-(2,4,5,7-tetrabromo-6-hydroxy-3-oxo-3*H*-xanthen-9-yl)- (Solvent Red 48)

Chemical Abstracts Service Registry Number
2134-15-8

Environment Canada
Health Canada

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Synopsis

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the Ministers of the Environment and of Health have conducted a screening assessment on Benzoic acid, 2,3,4,5-tetrachloro-6-(2,4,5,7-tetrabromo-6-hydroxy-3-oxo-3*H*-xanthen-9-yl)- (Solvent Red 48), Chemical Abstracts Service Registry Number 2134-15-8. This substance was identified as a high priority for screening assessment and included in the Ministerial Challenge because it had been 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 48 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 by Health Canada for categorization of substances on the Domestic Substances List. Therefore, this assessment focuses principally on information relevant to the evaluation of ecological risks.

Solvent Red 48 is an organic substance that may be used as a dye in Canada in various applications including in personal-care products and in drugs. Based on the survey conducted under section 71 of CEPA 1999, no companies reported importing or manufacturing the substance above the reporting threshold of 100 kg/year, and no use of the substance was reported above the reporting threshold of 1000 kg/year in 2006. However, two companies reported a stakeholder interest in Solvent Red 48.

Since there was no reporting of use, import or manufacture of Solvent Red 48 in Canada in 2006 at or above the reporting thresholds specified in the CEPA section 71 notice, releases of this substance to the Canadian environment are expected to be very low. As a conservative measure, it was assumed that 100 kg per year were used in both industrial and consumer use scenarios.

Based on available information, including a survey under section 71 of CEPA 1999, exposure of the general population to Solvent Red 48 from environmental media (ambient and indoor air, drinking water, soil, and sediment) is expected to be negligible. The general population of Canada may be exposed to Solvent Red 48 from use of certain cosmetics, including some personal care products, as it is an ingredient in some products on the Canadian market.

Solvent Red 48 is expected to have a high water solubility and a low octanol-water partition coefficient. It would be present in the environment primarily as a di-anion that is not volatile, is rather chemically stable, and is expected to have a tendency to partition to sediments if released to surface waters, and to remain in soils if released to land. This behaviour is mainly governed by electrostatic interactions.

Based on its physical and chemical properties, Solvent Red 48 is expected to be persistent in water, soil and sediment. With its relatively large molecular size and weight and low octanol-water partition coefficient, modelled results suggest that this substance is not bioaccumulative. The substance therefore meets the criteria for persistence in water, soil and sediment, but does not meet the criteria for bioaccumulation potential as set out in the *Persistence and Bioaccumulation Regulations*. In addition, newly identified experimental toxicity data for a chemical analogue, as well as new toxicity predictions that take into account revised estimates of bioaccumulation potential, suggest that the substance is likely to have a moderate to high potential for toxicity to sensitive aquatic organisms.

For this screening assessment, two very conservative exposure scenarios were considered involving both industrial use and consumer use, which result in discharges of Solvent Red 48 into the aquatic environment. The highest predicted environmental concentration in water, which was for the industrial use scenario, was almost two orders of magnitude below the predicted no-effect concentration calculated for sensitive aquatic biota.

Solvent Red 48 was not identified as posing a high hazard to human health. Based on consideration of the hazard profile of Solvent Red 48, upper-bounding estimates of exposure to cosmetics, including some personal care products containing this substance, and toxicokinetics of the substance, a concern for human health was not identified.

Based on the information available, it is concluded that Solvent Red 48 is not 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.

Based on the information available, it is concluded that Solvent Red 48 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. Solvent Red 48 meets the criteria for persistence, but does not meet the criteria for bioaccumulation potential as set out in the *Persistence and Bioaccumulation Regulations*.

This substance will be considered for inclusion in the *Domestic Substances List* inventory update initiative. In addition and where relevant, research and monitoring will support verification of assumptions used during the screening assessment.

Based on the information available, it is concluded that Solvent Red 48 does not meet any of the criteria set out in section 64 of the *Canadian Environmental Protection Act, 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 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 2006), 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 Benzoic acid, 2,3,4,5-tetrachloro-6-(2,4,5,7-tetrabromo-6-hydroxy-3-oxo-3*H*-xanthen-9-yl)- was identified as a high priority for assessment of ecological risk as it was 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 March 14, 2009 (Canada 2009). 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, submissions of information pertaining to the use of the substance were received.

Although Benzoic acid, 2,3,4,5-tetrachloro-6-(2,4,5,7-tetrabromo-6-hydroxy-3-oxo-3*H*-xanthen-9-yl)- was determined to be a high priority for assessment with respect to the environment, it did not meet the criteria for GPE or IPE. Therefore, this assessment focuses principally on information relevant to the evaluation of ecological risks.

Screening assessments focus on information critical to determining whether a substance meets the criteria 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 the additional 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 December 2009 for human health and ecological sections of the document. Key studies were critically evaluated; modelling results may have been used to reach conclusions.

When available and relevant, information presented in hazard assessments from other jurisdictions was considered. The 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 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.

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 the final assessment is based are summarized below.

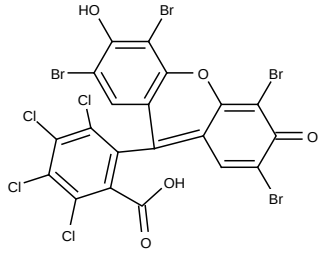
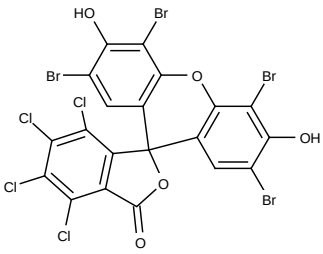
[1] A determination of whether one or more of the criteria of section 64 are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposures in the general environment. For humans, this includes, but is not limited to, exposures from ambient and 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 regulatory framework for the Workplace Hazardous Materials Information System [WHMIS] for products intended for workplace use.

Substance Identity

Substance Name

For the purposes of this document, this substance will be referred to as Solvent Red 48. Solvent Red 48 is defined by the Colour Index (CII 2002–2009) as a combination of multiple CAS numbers, including CAS RN 2134-15-8 and alternate CAS RN 13473-26-2 which represents the lactonic tautomer version of this substance (Table 1). However, for the purposes of the present report and the assessment of this substance, the common name Solvent Red 48 refers exclusively to the CAS RN 2134-15-8 which represents the quinonoid tautomer.

Table 1. Substance identity for Solvent Red 48

Chemical Abstracts Service Registry Number (CAS RN)	**2134-15-8**
DSL name	**Benzoic acid, 2,3,4,5-tetrachloro-6-(2, 4,5,7-tetrabromo-6-hydroxy-3-oxo-3H-xanthen-9-yl) -**
National Chemical Inventories (NCI) names[1]	*2',4',5',7'-Tetrabromo-4,5,6,7-tetrachlorofluorescein* (ECL) *2,3,4,5-Tetrachloro-6-(2,4,5,7-tetrabromo-6-hydroxy-3-oxo-3H-xanthen-9-yl)-benzoic acid* (ASIA-PAC, NZIoC)
Other names	*Acid Phloxine PB, C.I. 45410A; D and C Red No. 27; Fluorescein, 2',4',5',7'-tetrabromo-4,5,6,7-tetrachloro-; Phloxine BBN Supra; Phloxine O; Solvent Red 48; Tetrachlorotetrabromofluorescein; 2,4,5,7-Tetrabromo-12,13,14,15-tetrachloro-3,6-fluorandiol*
Chemical group (DSL Stream)	Discrete organics
Major chemical class or use	Dyes
Major chemical sub-class	Xanthene Dyes
Chemical formula	$C_{20}H_4Br_4Cl_4O_5$
Chemical structure	 Quinonoid tautomer  Lactonic tautomer
SMILES[2] (quinonoid	O=C/1\C(\Br)=C/2\Oc3c(Br)c(O)c(Br)cc3/C(/c3c(Cl)c(Cl)c(Cl)

tautomer)	c(Cl)c3C(=O)O)=C2/C=C1/Br
Molecular mass	785.68 g/mol

[1] National Chemical Inventories (NCI). 2007: ASIA-PAC (Asia-Pacific Substances Lists), ECL (Korean Existing Chemicals List); and NZIoC (New Zealand Inventory of Chemicals).

[2] Simplified Molecular Input Line Entry System.

Physical and Chemical Properties

As illustrated in Table 1, Solvent Red 48 is a free acid that exists in two tautomeric forms: the lactonic and quinonoid forms (Table 1). Tautomers are isomeric forms of a substance generally formed through delocalization of electrons and mobility of a group or atom which normally only occurs in liquid state or in solution and not in solid state. In Solvent Red 48, this occurs through delocalization of π-electrons in the xanthene group with the movement of a proton between a hydroxyl group and the carboxylic group formed by opening of the lactone (or cyclic ester group). It is expected that at pH 6–9, the soluble quinonoid would be the predominant form.

As a result of the ionizable nature of Solvent Red 48, Phloxine B (CAS RN 18472-87-2), the disodium salt of Solvent Red 48 (see Table 3), will serve as a suitable analogue for this substance (further justification for the suitability of this analogue can be found in the environmental fate section and in Appendix 3). In addition, through the review of the scientific literature, Rose Bengal (CAS RN 632-68-8) (see Table 3) was identified as being structurally similar to both Solvent Red 48 and Phloxine B as it also undergoes tautomerization between lactone and quinonoid forms. Rose Bengal and Phloxine B differ only in halogen substituents on the phenyl rings and in counter ion type. Rose Bengal contains four iodines and is a dipotassium salt, whereas Phloxine B has four bromines and is a disodium salt.

Table 2 contains experimental and modelled physical and chemical properties of Solvent Red 48 and its analogues that are relevant to its environmental fate. Chemical structures for Solvent Red 48 and the Rose Bengal and Phloxine B analogues are shown in Table 3. Key studies from which experimental data are available in the peer-reviewed open literature were critically reviewed for validity.

Models based on quantitative structure-activity relationships (QSAR) were used to generate data for some of the physical and chemical properties of Solvent Red 48. These models (except WSKOWWIN 2000) are mainly based on fragment addition methods, i.e., they rely on the structure of a chemical. Since these models only accept the neutral form of a chemical as input (in SMILES form), the modelled values shown in Table 2 are for the neutral quinonoid form of Solvent Red 48. Finally, the modelled log K_{ow} value for Solvent Red 48 (2.3) was determined using the experimental value adjustment (EVA) option in KOWWIN (2000). This approach estimates a log K_{ow} for a queried chemical (in this case Solvent Red 48) by comparing its structure to that of an analogue chemical that has an empirical log K_{ow} value (in this case Phloxine B). The empirical log K_{ow} value for

the analogue (i.e., -0.21) was adjusted by the model based on the influence that structural differences have on log K_{ow} when the two chemicals are compared.

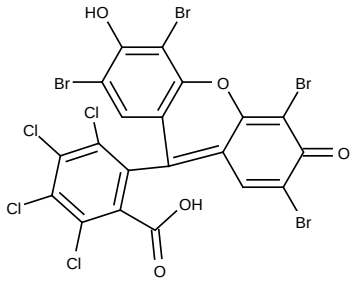
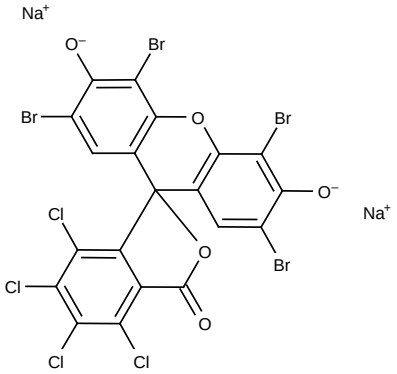
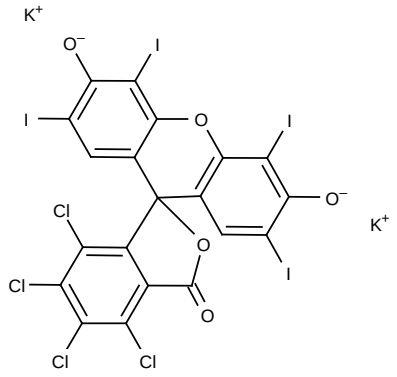
Table 2. Physical and chemical properties for Solvent Red 48, its sodium salt and a structural analogue

Property	Type	Value	Temperature (°C)	Reference
Melting point (ºC)				
Solvent Red 48	Modelled	324.9		MPBPWIN 2000
Rose Bengal (analogue)	Experimental	286–287		Amat-Guerri et al. 1990a
Boiling point (ºC)				
Solvent Red 48	Modelled	740.42		MPBPWIN 2000
Density (kg/m3)				
Solvent Red 48		No information Available		
Vapour pressure (Pa)				
Solvent Red 48	Modelled	2.826×10^{-17} (2.1197×10^{-19} mm Hg)	25	MPBPWIN 2000
Henry's Law constant (Pa·m^3/mol)				
Solvent Red 48	Modelled	1.10×10^{-15} (1.084×10^{-20} atm-m^3/mol)	25	HENRYWIN 2000
Log K_{ow} (Octanol-water partition coefficient) (dimensionless)				
Solvent Red 48	Modelled	2.30[6]		KOWWIN 2000
Phloxine B (Na salt of Solvent Red 48)	Experimental	-0.21 -0.74		Wang et al. 2006; Tonogai et al. 1982; Bergsten

Property	Type	Value	Temperature (°C)	Reference
		0.62		1995
Log K_{oc} (Organic carbon-water partition coefficient) (dimensionless)				
Solvent Red 48	Modelled	1.99[3]		PCKOCWIN 2000
Phloxine B (Na salt of Solvent Red 48)	Experimental	2.16[1] 2.28[2]		Li et al. 1998
Water solubility (mg/L)				
Phloxine B (Na salt of Solvent Red 48)	Experimental	90 000		MSDS 2006
pK_a (acid dissociation constant) (dimensionless)				
Solvent Red 48	Modelled	pK_{a1} = 1.32[5] pK_{a2} = 5[4]		ACD/pK_aDB 2005
Rose Bengal (analogue)	Experimental	pK_{a1} = 3.51[5] pK_{a2} = 4.05[4]		Martinez-Izquierdo et al. 1984

[1] Partition coefficients (K_{oc}) of Phloxine B determined in Kauai sediment.
[2] Partition coefficients (K_{oc}) of Phloxine B determined in Lihue silty clay.
[3] Log K_{oc} based estimation method using Log K_{ow} = 2.30.
[4] Value relates to equilibrium between carboxylic acid group and conjugate base.
[5] Value relates to equilibrium between phenolic group and conjugate base (π-stabilized).
[6] Modelled using experimental value adjustment (EVA) with Phloxine B empirical Log K_{ow} = -0.21.

Table 3. Structures of Solvent Red 48, Phloxine B (its sodium salt) and Rose Bengal (another structural analogue)

CAS RN	Common name (molecular weight in g/mol)	DSL name	Chemical structure
2134-15-8	Solvent Red 48 (785.68)	Benzoic acid, 2,3,4,5-tetrachloro-6-(2,4,5,7-tetrabromo-6-hydroxy-3-oxo-3H-xanthen-9-yl)-	
18472-87-2	Phloxine B (829.64)	Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 2',4',5',7'-tetrabromo-4,5,6,7-tetrachloro-3',6'-dihydroxy-, disodium salt	
632-68-8	Rose Bengal (1049.84)	Spiro[isobenzofuran-1(3H),9'-[9H]xanthen]-3-one, 4,5,6,7-tetrachloro-3',6'-dihydroxy-2',4',5',7'-tetraiodo-, dipotassium salt	

Sources

Solvent Red 48 is not known to be naturally occurring.

In response to a notice published under section 71 of CEPA 1999 (Environment Canada 2009a) there were no reports of import, manufacture or use of Solvent Red 48 in 2006 over the reporting thresholds. In addition, no reports of manufacture in or import into Canada of this substance at or above the reporting threshold of 100 kg in the 2005 calendar year were received in response to a notice published under section 71 of CEPA 1999 (Environment Canada 2009a). However, Declaration of Non-Engagement and/or Stakeholder Interest forms associated with this notice were received under both surveys mentioned above.

The quantity of Solvent Red 48 reported to be manufactured in, imported into, or in commerce in Canada during the calendar year 1986 was 100–1000 kg. The number of notifiers for the calendar years 1984–1986 was fewer than 4. Thus, the commercial activity for this substance in Canada appears to have decreased between 1986 and 2006.

Uses

Internationally, and in Canada, Solvent Red 48 is used as a component in colouring agents in personal care products and in drugs (US FDA 1982a, 2009; European Commission 2009; CNS 2009). One stakeholder who expressed an interest in this substance noted that Solvent Red 48 is imported as part of a cosmetic formula (Environment Canada 2009a). In the mid-1980s, the following DSL use codes were identified for Solvent Red 48: 13 - Colourant - pigment/stain/dye/ink; 60 – Cosmetics.

In Europe, Solvent Red 48 is listed in the Cosmetics Directive Annex IV Part 1 as "CI 45410" (and associated CAS RNs 13473-26-2, 18472-87-2) permitting its use as a colourant in all cosmetic products (European Commission 2009). In the United States, Solvent Red 48 (as D&C Red 27 or D&C Red 28) is permitted for use as a colourant in drugs and cosmetics, with the exception of eye-makeup formulations (US FDA 1982a; Lipman 1995; NTP 2000). Retail products containing Solvent Red 48 in the United States were reported to be primarily lipsticks and blushers (NTP 2000). Neither Solvent Red 48 nor any of its associated names, i.e., D&C Red 27, Red 27, Phloxine B, D&C Red 28, or Red 28 (Appendix 3), is currently listed on Health Canada’s Cosmetic Ingredient Hotlist (Health Canada 2009). Meanwhile, Solvent Red 48 is found in Health Canada’s cosmetics notification system (CNS) database as an ingredient in approximately 800 cosmetics, including some personal care products (CNS 2009). Its listing, however, is not only under the name “Solvent Red 48,” but also by its Color Index Code: CI 45410, Red 27 and Red 28, which not only contain CAS RN 2134-15-8 but also CAS RN 13473-26-2 and CAS RN 18472-87-2 (Appendix 3).

In Canada, Solvent Red 48 is listed in the *Food and Drug Regulations* in section C.01.040.2 (3)(*a*) as a colouring agent permitted in drugs for internal and external use under the name Phloxine B Acid Form (D&C Red No. 27; C.I. No. 45410:1) Canada [1978]). Thus, this colouring agent is permitted in pharmaceutical drugs, natural health products and veterinary drugs. It is also listed as an acceptable non-medicinal ingredient in the Natural Health Products Ingredients Database (LNHPD 2009; NHPID 2010).

Solvent Red 48 is not listed in Table III of Division 16 of the *Food and Drug Regulations* as a food additive permitted for use as food colours, nor is it approved for any other food additive use in Canada (Canada 1978). A submission for the use of Solvent Red 48 in food packaging materials or in formulations of incidental additives has never been received at Health Canada (2010 Personal communication from Food Directorate, Health Canada to Existing Substances Risk Assessment Bureau, Health Canada).

Solvent Red 48 is not identified as a formulant or active ingredient in pest control products under the Registered Product Database (PMRA 2007, 2008).

Releases to the Environment

In principle, the releases of Solvent Red 48 to the environment depend upon various losses of the substance from its manufacture, industrial use, and/or consumer/commercial use. These losses can be grouped into seven types: (1) discharge to wastewater; (2) emission to air; (3) loss to paved/unpaved land surfaces; (4) chemical transformation; (5) disposal to landfill; (6) disposal by recycling; and (7) disposal by incineration.

However, since there were no reports of use, import or manufacture of Solvent Red 48 in Canada in 2006 at or above the reporting thresholds specified in the CEPA section 71 notice (Environment Canada 2009a), releases of this substance to the Canadian environment are expected to be very low.

This substance is expected to be found in some consumer products. Based on notifications in Health Canada's CNS, Solvent Red 48 is present in approximately 800 cosmetics, including some personal care products (CNS 2009). It is anticipated that releases from such products would be widespread but low. Available information is currently not sufficient to derive a quantitative estimate for these releases, however.

The threshold of 100 kg was used throughout this screening assessment to capture the maximum potential mass of this substance in use in Canada that would be below the threshold reporting value.

Environmental Fate

As shown in Table 2, Solvent Red 48 has two dissociation constants (pK_a), indicating that the neutral molecular form will be the dominant form only at a pH below 1.32. Under

these acidic conditions, it has been found that the lactonic tautomer is favoured. However, at higher pHs, the di-ionic quinonoid (i.e., ring-opened and ionized carboxylate) form is dominant, as evidenced by the lack of detection of the di-ionic lactone form of Rose Bengal analogue by Amat-Guerri et al. (1990b). In addition, the anionic (phenolic group) quinonoid would be more prevalent due to the higher stability brought about by the available delocalization of the negative charge of the phenolic groups via the xanthene π-system in the quinonoid structure versus the lactone structure where this delocalization is not possible (Amat-Guerri et al. 1990b). It is therefore expected that at environmentally relevant pH (6–9), the soluble, dianionic quinonoid would be the predominant form present (both pK_a values are ≤ 5). As a result, the partitioning of the ionized form of the analogue Phloxine B is expected to be directly indicative of the partitioning behaviour of Solvent Red 48 in aqueous media at environmentally relevant pH (Table 3; analogue data). Both Phloxine B and Solvent Red 48 will yield similar ionized structures in the environment.

In a study of the identification and measurement of certain dyes in a municipal wastewater treatment plant, the three xanthene dyes tested, including Phloxine B, were found exclusively in the solids component of the wastewater (i.e., sludge) (Borgerding and Hites 1994). The authors of this study also conclude that partitioning between the solid and liquid phases may be dependent on ionic interactions between the dyes and sludge and/or on simple hydrophobic equilibrium processes. Thus, despite the low predicted log K_{ow} and log K_{oc} of 2.30 and 1.99 for Solvent Red 48, the charged nature of this substance may lead to a greater partitioning to soil and sediment than predicted from hydrophobicity alone—due to electrostatic interactions. This is supported by the results of Li et al. (1998), who measured the concentration of two structurally similar xanthene dyes, including Phloxine B, at a spill site. They found that the concentrations in sediment were much higher than in water, with the sediment to water ratio increasing over time from around 20 to 2600 from day 12 to 123 (Li et al. 1998). In this study, it was also observed in a batch experiment with sediment and solids that adsorption coefficients (see log K_{oc} in Table 2) of Phloxine B were somewhat higher in silty clay soil than in sediments, although the organic carbon content of the sediment is higher than the soil. The increased adsorptive capacity of the soil for these dyes could be attributed not only to organic carbon partitioning, but also to electrostatic interactions with the mineral phases of the soil. Thus, considering that at environmental pH in aqueous media both Phloxine B and Solvent Red 48 are expected to be present in essentially the same anionic form, and given that the experimental log K_{oc} value of 2.16–2.28 for the Phloxine B analogue is quite similar to the modelled value for Solvent Red 48 (1.99), upon release to water Solvent Red 48 (like Phloxine B) is expected to have a tendency to partition into the sediments with some of it remaining in the aqueous phase.

Due to the expected high water solubility of Solvent Red 48, along with its tendency to form the dianionic quinonoid identical to Phloxine B in water at environmentally relevant pH if released to soil, some Solvent Red 48 would be expected to remain in the soil, with some leaching after partitioning into soil pore water also expected.

Persistence and Bioaccumulation Potential

Environmental Persistence

No environmental monitoring data relating to the presence of Solvent Red 48 in the Canadian environment (air, water, soil or sediment) have been identified. According to the Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers (ETAD 1995), dyes are, with some exceptions, considered essentially non-biodegradable under aerobic conditions. Repeated evaluation of ready and inherent biodegradability using accepted screening tests (see Organisation for Economic Co-operation and Development Guidelines for Testing Chemicals) have confirmed this understanding (Pagga and Brown 1986; ETAD 1992). Based on the chemical structure of Solvent Red 48 (i.e., aromatic halogens), there is no reason to suspect that biodegradation will be other than that described for dyes in general (ETAD 1995).

Xanthene dyes are photoreactive and the presence of halogens has been found to increase their photoreactivity by increasing the efficiency of their transition to the excited triplet state (Walthall and Stark 1999). Wang et al. (2006) showed that with an increasing number of halogen substituents on xanthene dyes, the singlet oxygen yields increased. Once in the excited triplet state, the dye is able to excite an oxygen molecule, which can cause degradation of the dye by attacking the double bonds in the dye molecule (Heitz 1995). Empirical data for analogue Phloxine B (See Table 4a) show that photolytic degradation proceeds quickly for this substance in solution (< 1 day). Wang et al. (1998) demonstrated that photodegradation half-lives for Phloxine B in tap, stream or sea water (pH 7.2, 6.9 and 7.9, respectively) ranged from 10 to 26 minutes under sunlight exposure at ambient air temperatures in Hawaii (August–October).

Table 4a. Empirical data for photodegradation and dissipation of Phloxine B analogue

Medium	Fate process	Value	Degradation endpoint/units	Reference
Water	Dissipation[1]	< 12	Half-life, days	Li et al. 1998
Water	Photodegradation	< 1	Half-life, days	Tonogai et al. 1979
Water	Photodegradation	< 1	Half-life, days	Wang et al. 1998
Air	Photodegradation	< 1	Half-life, hours	Heitz and Wilson 1978
Soil	Dissipation[1]	< 7	Half-life, days	Alcantara-Licudine et al. 1999
Sediment	Dissipation[1]	123–284	Half-life, days	Li et al. 1998

[1]Dissipation studies and half-lives calculated from such studies are based on a mass-balance of the substance in an environmental compartment. Half-lives are calculated based on possible losses from transformation and sorption (matrix binding). The half-lives reported therefore do not represent the intrinsic stability of the compound alone and cannot easily be compared to regulatory persistence criteria that are based on intrinsic stability.

Alcantara-Licudine et al. (1999) studied the dissipation of Phloxine B in soil following its aerial spray on a coffee field in Hawaii. The mixture was composed of 0.68% Phloxine B, such that 11.1 g of the substance was applied per acre in each spray. The field was sprayed weekly for ten weeks. The soil was a silty clay loam with a slight to moderate acidity (pH < 6), suggesting that sorption might be slightly stronger in this soil compared to a more neutral soil. The dissipation half-life of Phloxine B was approximately seven days (Table 4a). The concentration of Phloxine B was also measured at 5–10 cm depth in the soil, and was found to be an order of magnitude lower than the topmost layer, suggesting that the loss in the upper layer is mostly due to degradation and not leaching. The concentrations of Phloxine B in the lower layer also appeared to decrease over time. Based on the results of this study along with the experimentally determined aqueous photodegradation half-lives of < 1 day (Table 4a), it would be expected that Solvent Red 48 would also undergo photolysis in the topmost layers of soil.

Li et al. (1998) also measured the concentration of Phloxine B in sediment, following a spill. As shown in Table 4a, Li et al. (1998) observed longer dissipation half-lives for Phloxine B in sediment relative to water. The longer half-life may be a result of reduced light, although the depth of the water was only 8–10 cm and sediment samples were taken at a depth of 5 cm. The authors of this study also note, however, that some of the observed loss of Phloxine B may be due to washout by rain. Thus, there remains some uncertainty in the significance of these data in relation to the degradation half-life of Solvent Red 48 in water, soil and sediment.

All of the empirical data relate to primary photolysis-type degradation reactions, which would not be expected to be effective in deeper soils, sediments and water where light cannot penetrate. Wang et al. (1998), for example, observed that Phloxine B in water was stable in the dark up to a few weeks, although the data were not presented and the half-lives were not calculated, as the study focused primarily on the photolysis of Phloxine B.

In an assessment of Phloxine B in insecticide application trials, Bergsten (1995) also conclude that Phloxine B was not anticipated to persist for very long in the environment and rapid degradation of these dyes in water through photobleaching has been demonstrated.

Although experimental data on the dissipation of the disodium salt of Solvent Red 48 (i.e., Phloxine B) are available, half-lives calculated are based on possible losses from transformation and sorption. The half-lives reported therefore do not represent the intrinsic stability of the compound and cannot easily be compared to regulatory persistence criteria that are based on intrinsic stability. Thus, a QSAR-based weight-of-evidence approach (Environment Canada 2007) was also applied to understand the intrinsic stability of Solvent Red 48. Given the ecological importance of the water

compartment, the fact that most of the available models apply to water, and the fact that Solvent Red 48 is expected to be released to this compartment, biodegradation in water was primarily examined using the degradation models shown in Table 4b. This substance contains almost fully halogenated (i.e., chlorine and bromine) aromatic groups, which may lead to a less biodegradable compound.

Table 4b summarizes the results of available QSAR models for degradation in water and air.

Table 4b. Modelled data for degradation of Solvent Red 48

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
AIR			
Atmospheric oxidation	AOPWIN 2000[1]	$t_{1/2}$ = 0.347 days	< 2
Ozone reaction	AOPWIN 2000[1]	$t_{1/2}$ = 0.168 days	< 2
Primary biodegradation			
Biodegradation (aerobic)	BIOWIN 2000[1] Sub-model 4: Expert Survey (qualitative results)	1.9[3] "biodegrades relatively slowly"	potentially ≥ 182
Ultimate biodegradation			
Biodegradation (aerobic)	BIOWIN 2000[1] Sub-model 3: Expert Survey (qualitative results)	0.49[3] "biodegrades slowly"	≥ 182
Biodegradation (aerobic)	BIOWIN 2000[1] Sub-model 5: MITI linear probability	-0.31[4] "biodegrades very slowly"	≥ 182
Biodegradation (aerobic)	BIOWIN 2000[1] Sub-model 6: MITI non-linear probability	0.00[4] "biodegrades very slowly"	≥ 182
Biodegradation (aerobic)	TOPKAT 2004 Probability	n/a[2]	n/a
Biodegradation (aerobic)	CPOPs 2008 % BOD (biological oxygen demand)	% BOD = 1.9 "biodegrades very slowly"	≥ 182

[1] EPIsuite (2007).
[2] Model does not provide an estimate for this type of structure.
[3] Output is a numerical score from 0 to 5.
[4] Output is a probability score.

The half-life value in Table 4b for TOPKAT is not considered to be reliable, as no chemicals of structural comparability are contained in the model's training set. In addition, the results for CPOPs are considered borderline unreliable, since less than 60% of the structure of Solvent Red 48 was within the structural domain of the model. Nonetheless, other important domains of the model were satisfied (e.g., metabolic and parametric domains), and the CPOP result is consistent with other model predictions and is consistent with what would be expected for this chemical structure (highly substituted halogenated aromatic) and stability properties that make it useful as a dye.

In air, a predicted atmospheric oxidation half-life value of < 2 days for gas phase Solvent Red 48 (see Table 4b) demonstrates that this substance may be rapidly oxidized, although

due to the very low modelled vapour pressure and Henry's Law constant for Solvent Red 48, the atmosphere is not a significant environmental compartment for this substance.

The modelled data in Table 4b, as well as structural considerations, suggest that the biodegradation of Solvent Red 48 is very slow and the half-life in water would be ≥ 182 days. The results provided in Table 4a for Phloxine B, a disodium salt (analogue) of Solvent Red 48, cannot easily be interpreted in relation to the intrinsic stability of the substance. Compounds of this type are expected to undergo photodegradation in light-penetrating conditions (i.e., surface water, soil and sediments), but the identities of their degradation products are not known. Furthermore, such compounds will likely remain stable when light is absent or limited (e.g., in subsurface soil or buried sediment). Therefore, the biodegradation half-life estimates in Table 4b are considered better measures of the intrinsic stability of the substance.

Using an extrapolation ratio of 1:1:4 for a water: soil: sediment biodegradation half-life (Boethling et al. 1995), and the ultimate degradation half-life of ≥ 182 days in water, the half-life in soil is also ≥ 182 days and the half-life in sediments is ≥ 365 days. This indicates that Solvent Red 48 is expected to be persistent in soil and sediment.

Therefore, based on the consistency of data in Table 4b as well as structural considerations suggesting increased stability, Solvent Red 48 meets the persistence criteria in water, soil and sediment (half-lives in soil and water ≥ 182 days and half-life in sediment ≥ 365 days) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential for Bioaccumulation

Since no experimental bioaccumulation factor (BAF) and/or the bioconcentration factor (BCF) data for Solvent Red 48, its disodium salt analogue (Phloxine B) or Rose Bengal were available, a predictive approach was applied using available BAF and BCF models as shown in Table 5 below. According to the *Persistence and Bioaccumulation Regulations* (Canada 2000), a substance is bioaccumulative if its BCF or BAF is ≥ 5000. However, measures of the BAF are the preferred metric for assessing bioaccumulation potential of substances. This is because the BCF may not adequately account for the bioaccumulation potential of substances via the diet, which predominates for substances with log K_{ow} >~ 4.0 (Arnot and Gobas 2003). Kinetic mass-balance modelling is, in principle, considered to provide the most reliable prediction method for determining the bioaccumulation potential because it can allow for competing rates of uptake and elimination, such as metabolic biotransformation. This is provided that the substance is within the domain of the model. Current mass balance models may not adequately address substances that ionize significantly in the environment (Arnot and Gobas 2003).

BCF and BAF estimates were generated using the Arnot-Gobas mass balance model (Arnot and Gobas 2003). Mitigation of bioaccumulation via gut biotransformation was not relevant to consider because, given Solvent Red 48's relatively low estimated log K_{ow} (2.30), uptake and loss will be mainly a function of gill transfer and not the diet.

Table 5. Modelled data for bioaccumulation for Solvent Red 48

BCF[1] (L/kg)	BAF[1] (L/kg)	Reference
12.52	12.53	Arnot and Gobas 2003 (Gobas BCF/BAF Middle Trophic Level)
3.16	n/a	BCFWIN 2000
6.90	n/a	CPOPs 2008

[1]Based on log K_{ow} of 2.3 modelled for Solvent Red 48.

One of the modelled values in Table 5 (CPOPs 2008) is considered less reliable, as few chemicals of structural comparability are contained in its training set (only 33% within the structural domain of the model). In addition, the Gobas BCF/BAF model may not provide reliable predictions for ionizable substances. The BCF value in Table 5 (BCFWIN 2000) and log K_{ow} estimate of 2.3 nevertheless suggest that Solvent Red 48 would not have a tendency to bioaccumulate.

Recent investigations relating fish BCF data and molecular size parameters by Dimitrov et al. (2002), Dimitrov et al. (2005) and the Baseline Bioaccumulation Model (BBM 2008) 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 falls appreciably when maximum diameter is greater than ~ 1.5 nm and falls more significantly when molecules have a maximum diameter of > 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}) of > 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. As Arnot et al. (2010) point out, 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.

The maximum diameter (D_{max}) for Solvent Red 48 is estimated at 1.4–1.5 nm with an average effective diamerter (D_{eff}) of 1.3 nm which approaches or exceeds some of the

values cited above and suggest that the uptake rate of this substance may be slower compared to that of smaller, more compact substances, thus mitigating the overall bioconcentration potential.

The available evidence indicates that Solvent Red 48 is expected to have low bioaccumulation potential due to its physical and chemical properties (i.e., low lipophilicity, ionic character as well as relatively high molecular weight and cross-sectional diameter). Predicted BCF and BAF values are much less than the bioaccumulation criterion (BCF or BAF $\geq$ 5000) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000), and are consistent with what would be expected for ionic chemicals. Therefore, Solvent Red 48 is not considered to be bioaccumulative according to these criteria.

Potential to Cause Ecological Harm

Ecological Effects Assessment

A – In the Aquatic Compartment

There is modelled and experimental evidence that Solvent Red 48 may cause harm to aquatic organisms following short-term (acute) exposure at relatively low concentrations (< 1 mg/L). Although empirical toxicity information is available (Table 7a) for the salt of Solvent Red 48 (Phloxine B), modelled predictions for aquatic toxicity were also made for Solvent Red 48 (Table 7b). Lipman (1995) has suggested that the United States Food and Drug Administration (U.S. FDA) regards Solvent Red 48 as toxicologically equivalent to Phloxine B, its disodium salt.

Table 7a. Empirical data for aquatic toxicity of the analogue, Phloxine B

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Daphnia pulex	Acute (48 hours)	LC_{50}[1]	0.423	Walthall and Stark 1999
Daphnia pulex	Chronic (10-day)	LOEC[4,6]	1.00	Walthall and Stark 1999
Daphnia pulex	Chronic (10-day)	NOEC[5,6]	1.00	Walthall and Stark 1999
Japanese Medaka (*Oryzias latipes*)	Acute (48 hours)	TLm[2]	190	Tonogai et al. 1978
Japanese Medaka (*Oryzias latipes*)	Acute (48 hours)	TLm[2]	200	Tonogai et al. 1979
Japanese Medaka (*Oryzias latipes*)	Acute (48 hours)	TLm[2,3]	60	Tonogai et al. 1979
Japanese Medaka (*Oryzias latipes*)	Acute (48 hours)	TLm[2]	200	Tonogai et al. 1982

[1] LC_{50} – The concentration of a substance that is estimated to be lethal to 50% of the test organisms.
[2] TLm – Median Tolerance Limit: the concentration of substance at which just 50% of the test organisms are able to survive for a specified period of exposure (equivalent to LC_{50}).
[3] Following 10 hours of irradiation using a high-pressure mercury lamp.
[4]LOEC – The Low Observed Effect Concentration is the lowest concentration in a toxicity test that caused a statistically significant effect in comparison to the controls.
[5]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.
[6]Reporductive potential as measured by mean number of offspring per surviving individual.

The toxicity of xanthene dyes has been observed to increase following photoirradiation. This increased toxicity is expected to occur as a result of their excitation to triplet state where the dye is able to excite an oxygen molecule, which can react with and cause damage to biomolecules (Walthall and Stark 1999; Tonogai et al. 1979). Walthall and Stark (1999) observed the effect of the exposure of fluorescent light to Phloxine B on its acute mortality to *Daphnia* over time. They reported a 48-hr LC_{50} of 0.423 mg/L; the lowest concentration reported to significantly affect the reproductive potential of *Daphnia* neonates in a chronic (10-day) study was 1 mg/L. Walthall and Stark (1999) also found that under normal test conditions under florescent lights with a 16 hour / 8 hours light / dark regime after three days, the residues were no longer significantly toxic to newly exposed neonates, which was attributed to photolytic degradation of the compound.

Since the empirical data relate to a close analogue and not to Solvent Red 48 (CAS RN 2134-15-8) itself, the ecotoxicity of Solvent Red 48 was also investigated using models; the results are shown in Table 7b.

Table 7b. Modelled data for aquatic toxicity

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Fish	Acute (96 hours)	LC_{50}[1]	337[3]	ECOSAR 2004[4]
			3.55	AIEPS 2003–2007
Daphnia	Acute (48 hours)	LC_{50}[1]	202[3]	ECOSAR 2004[4]
			0.83	AIEPS 2003–2007
Algae	Acute (96 hours)	EC_{50}[2]	98[3]	ECOSAR 2004[4]
	Acute (72 hours)	EC_{50}[2]	5.91	AIEPS 2003–2007

[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 effect on 50% of the test organisms.
[3] Estimate using modelled log K_{OW} (2.3) for Solvent Red 48.
[4] Neutral organic structure-activity relationship (SAR).

Some of the modelled data indicate that, for *Daphnia*, the acute toxicity may be high (acute $LC/EC_{50} \leq 1.0$ mg/L). The AIEPS-modelled values were considered borderline reliable, as few of the structural analogues contained in the training set were similar to Solvent Red 48, and in fact only for the fish (Fathead Minnow - *Pimephales promelas*) LC_{50} value were the top five analogues between 70 and 80% similar to Solvent Red 48. In addition, Solvent Red 48 was not within the domain of applicability for CPOPs (2008),

and thus no results were available for this model. However, this model did identify certain structural aspects as likely biologically reactive, although with an unknown mode of action.

As stated previously, it is expected that at environmentally relevant pH (6–9), the soluble, dianionic quinonoid would be the predominant form of Solvent Red 48 present. As a result, the ionized form of the analogue Phloxine B is expected to be toxicologically equivalent to Solvent Red 48 in aqueous media at environmentally relevant pH. Thus, it is expected that the empirical toxicity information available for Phloxine B would be suitable read-across data for Solvent Red 48 in the aqueous environment.

The weight of evidence regarding experimental and modelled data indicates that Solvent Red 48 may cause acute harm to sensitive aquatic organisms at low concentrations (i.e., acute LC_{50}s are < 1.0 mg/L).

B – In Other Environmental Compartments

No suitable ecological effects studies were found for this compound or its analogues in media other than water.

When Solvent Red 48 is released into a water body, it partitions into suspended particulate matter and to bottom sediments, where sediment-dwelling organisms would be exposed to the substance. However, no environmental monitoring data or toxicity data specific to sediment-dwelling organisms are available for this substance.

Ecological Exposure Assessment

No data concerning concentrations of this substance in water in Canada have been identified. Therefore, environmental concentrations are estimated from available sources, including estimated substance quantities, release rates, and size of receiving water bodies. Considering that there were no reports of use, import or manufacture of Solvent Red 48 in Canada in 2006 at or above the reporting thresholds specified in the CEPA section 71 notice (Environment Canada 2009a), releases of this substance to the Canadian environment are expected to be very low. As a conservative measure, it was assumed that 100 kg/yr was used in both industrial and consumer use scenarios.

A – Industrial Release

The aquatic exposure of Solvent Red 49 is expected if the substance is released from industrial use to a wastewater treatment plant and the treatment plant discharges its effluent to a receiving water body. The concentration of the substance in the receiving water near the discharge point of the wastewater treatment plant is used as the predicted environmental concentration (PEC) in evaluating the aquatic risk of the substance. It can be calculated using the equation

$$C_{water-ind} = \frac{1000 \times Q \times L \times (1-R)}{N \times F \times D}$$

where

$C_{water-ind}$:	aquatic concentration resulting from industrial releases, mg/L
Q:	total substance quantity used annually at an industrial site, kg/yr
L:	loss to wastewater, fraction
R:	wastewater treatment plant removal rate, fraction
N:	number of annual release days, d/yr
F:	wastewater treatment plant effluent flow, m^3/d
D:	receiving water dilution factor, dimensionless

As Solvent Red 48 may be used industrially and is expected to be released to water, a worst-case industrial release scenario is used to estimate the aquatic concentration of the substance with the help of Environment Canada's (2009b) Industrial Generic Exposure Tool – Aquatic (IGETA). The scenario is made conservative by assuming that the maximum potential quantity of the substance under the section 71 reporting threshold (i.e., 100 kg) is used by Canadian industry at one hypothetical industrial facility, and that the loss to sewer water is high (5% of the total quantity, resulting from the cleaning of chemical containers and process equipment). The scenario also assumes that the release occurs 250 days per year, typical for small and medium-sized facilities, and is sent to a local sewage treatment plant (STP) with a zero removal rate for the substance. Upon combining with the STP effluent, the small receiving water body is assumed to have an actual or equivalent flow of 34 560 m^3 per day. Based on the above assumptions, and a total quantity of 100 kg/yr for industrial use, an aquatic concentration of 0.0006 mg/L was calculated (Environment Canada 2009c).

B – Consumer Release

As Solvent Red 48 is found in some consumer products and can be released to water, Mega Flush, Environment Canada's spreadsheet tool for estimating down-the-drain releases from consumer uses, was employed to estimate the potential substance concentration in multiple water bodies receiving STP effluents into which consumer products containing the substance may have been released (Environment Canada 2009d).

The spreadsheet tool is designed to provide these estimates based on conservative assumptions regarding the amount of the substance used and released by consumers.

By default, it was assumed that the primary and secondary STP removal rates are 0%, losses from use are 100%, consumer use of the substance is 365 days/year, and the flow rate at all sites is relatively low (i.e., the tenth percentile of annual flow values). These estimates are made for approximately 1000 release sites across Canada, which account for most of the major STPs in the country.

The equation and inputs used to calculate the predicted environmental concentration (PEC) of Solvent Red 48 in the receiving water bodies are described in Environment Canada (2009e). The scenario was run assuming a total consumer use quantity of 100 kg/yr (based on the section 71 reporting threshold).

Using this scenario, the tool estimates that the PEC in the receiving water bodies ranges from 2.7×10^{-6} to 1.5×10^{-4} mg/L.

Characterization of Ecological Risk

The approach taken in this ecological screening assessment was to examine various supporting information and develop conclusions based on a weight-of-evidence approach and using precaution as required under CEPA 1999. Lines of evidence considered include results from a conservative risk quotient calculation, as well as information on persistence, bioaccumulation, toxicity, sources and fate of the substance.

Solvent Red 48 is expected to be persistent in water, soil and sediment, and it is expected to have a low bioaccumulation potential. Since there were no reports of import, manufacture or use of Solvent Red 48 in 2006 over 100 kg/yr, releases of this substance to the Canadian environment are expected to be very low. However, this substance may possibly cause acute harm to sensitive aquatic organisms at low concentrations (i.e., acute LC_{50}s are < 1.0 mg/L). Given the substance's use pattern, the main environmental compartment for release is water. If released to water, a portion of the substance will partition to sediment.

A risk quotient analysis, integrating conservative estimates of exposure with toxicity information, was performed for the aquatic medium to determine whether there is potential for ecological harm in Canada. The generic scenario described above yielded the more conservative (highest) PEC of 0.0006 mg/L (Environment Canada 2009c). A predicted no-effect concentration (PNEC) was derived from the acute LC50 toxicity value of 0.423 mg/L for *Daphnia* (see Appendix 1 for robust study summary of Walthall and Stark 1999), by dividing this value by an assessment factor of 10 (to account for inter-species and intra-species variability in sensitivity and to estimate a long-term no-effects concentration from a short-term LC_{50}) to give a value of 0.042 mg/L. A higher assessment factor (e.g., 100) was not used because of the empirical and model evidence suggesting that most organisms are less sensitive than *Daphnia*, and the empirical data

(Walthall and Stark 1999) suggesting that *Daphnia* are more sensitive to acute than to chronic (longer-term) exposures. The resulting risk quotient (PEC/PNEC) = 0.014. Therefore, harm to aquatic organisms is unlikely.

For this substance, a risk quotient based on exposure in sediment pore water could have been calculated. In the calculation, bottom sediment and pore water would be assumed to be in equilibrium with the overlying water, and benthic and pelagic organisms would be assumed to have similar sensitivities to the substance. Therefore, the PEC and PNEC for pore water would be the same as for the aquatic compartment. This equilibrium approach would thus result in a risk quotient (PEC/PNEC) for the sediment compartment that is the same as for the aquatic compartment.

This information suggests that Solvent Red 48 does not have the potential to cause ecological harm in Canada.

Uncertainties in Evaluation of Ecological Risk

Given the use of this substance in other countries, it is expected that the substance is entering the Canadian market as a component of manufactured items and consumer products. Available information is currently not sufficient to derive a quantitative estimate that would help determine the importance of this source. However, information obtained from the section 71 survey and other sources indicates that it is present in a number of products imported into Canada. The 100-kg mass used throughout this screening assessment (equal to the section 71 survey reporting threshold) is intended to capture a high-end potential quantity of this substance in use in Canada.

Phloxine B, a salt of Solvent Red 48, was used as an analogue throughout the ecological assessment section of this assessment. However, as it is a salt, it is expected to have slightly different physical and chemical properties, which creates uncertainty in the predictions of fate, persistence, bioaccumulation potential and aquatic toxicity that are based on these properties. Nevertheless differences between the properties and environmental behaviour of Phloxine B and Solvent Red 49 are expected to be small.

Regarding ecotoxicity, based on the predicted partitioning behaviour of this chemical, the significance of soil and sediment as important media of exposure is not well addressed by the available effects data. Indeed, the only effects data identified apply primarily to pelagic aquatic exposures, although the water column may not be the only medium of concern based on partitioning estimates.

It should be noted that Solvent Red 48 is suspected of undergoing protein conjugation (binding) via Michael Type nucleophilic addition (quinone fragment) as well as nucleophilic substitution of halo-aromatics. These two mechanisms are associated with skin sensitization occurring as a result of covalent adduct formation at an electrophilic center. While these types of substitutions are often mitigated in many non-ionic dyes due to lack of bioavailability, Solvent Red 48 and its sodium salt Phloxine B have sufficient

water solubility to suggest that low bioavailability may not mitigate protein binding potential. Therefore, the mechanism of bioaccumulation and toxicity of this class of ionic dyes may not occur via classic passive-diffusion principles. It may in fact occur via binding to blood albumin or muscle tissues from facilitative transport uptake processes (i.e., transmembrane carrier proteins). The bioaccumulation and toxicity potential may thus be underestimated by current models, which can accommodate passive-diffusion principles but not other active mechanisms. Empirical *in vitro* and *in vivo* evidence would be required to demonstrate protein binding potential and the resulting potential for bioaccumulation and excess toxicity above the narcotic baseline based on passive diffusion.

Potential to Cause Harm to Human Health

Solvent Red 48 (CAS RN 2134-15-8) is a xanthene dye in the fluorescein sub-family. It undergoes a complex ionization process involving various charged and uncharged forms of possible tautomers (Amat-Guerri et al. 1990b). A simplified schematic of structures and their associated names and CAS RNs are presented in Appendix 3 (Amat-Guerri et al. 1990b; US FDA 1982c; Levillain and Fompeydie 1985; Zuckerman 1974).

The tautomeric forms of Solvent Red 48 (the lactonic and quinonoid acid forms) exhibit different physical and chemical properties. In its neutral lactone form, it is colourless and insoluble in water, while the quinonoid acid form can undergo ionization of the phenol and carboxylic acid groups resulting in the soluble mono- and di-anionic quinonoid species. Therefore, the specific molecular species present in environmental media or consumer products is dependent largely on the pH of the medium. Given the acid-base equilibrium for these species, the di-anionic quinonoid form is expected to be dominant under conditions of environmentally and physiologically relevant pH. The sodium salt, Phloxine B, is also expected to fully dissociate in aqueous media resulting in the dominant quinonoid di-anion.

The complex ionization chemistry is reflected in the multiple names and CAS RNs associated with this substance. In particular, CAS RN 2134-15-8 is listed in SciFinder only as an alternate for CAS RN 13473-26-2 under the Color Index CI name Solvent Red 48, as well as D&C Red No.27 (acid). The sodium salt is listed under the names Phloxine B, CI name Acid Red 92, or D&C Red No. 28 (sodium salt) with CAS RN 18472-87-2 and its alternate CAS RN of 4618-87-2. In addition, it is also listed as a “component” of CAS RN 13473-26-2. The U.S. FDA considered D&C Red No.27 and D&C Red No.28 to be toxicologically equivalent for the purposes of registration.

Considering the complex nature of the chemistry associated with its structure (Appendix 3), all relevant data for Solvent Red 48, including Phloxine B, as well as the associated CAS RNs and names, have been considered in the human health assessment.

Exposure Assessment

Based on the use pattern and physical and chemical properties of Solvent Red 48, exposure to the general population of Canada via environmental media is expected to be negligible (ChemCAN 2003).

Exposure to Solvent Red 48 from use of cosmetics, including some personal care products, was estimated using ConsExpo 4.1 (ConsExpo 2006) for the products found in the CNS database (CNS 2009) and the results are summarized in Table 8. The details of exposure scenarios are provided in Appendix 4a for the oral route and Appendix 4b for the dermal route.

For frequently used products, such as skin moisturizer, facial and eye makeup, lipstick, skin cleanser, and fragrance, upper-bounding estimates of chronic exposure were derived for each product use, as well as the total exposure estimate for use of multiple products identified as containing Solvent Red 48. Use of lipstick was identified to be the only source among the reported products leading to chronic oral exposure, and the upper-bounding estimate from this use was derived to be 0.2 mg/kg-bw per day. For dermal route, the predominant contribution to total exposure was the use of skin moisturizer (2.0 mg/kg-bw per day), followed by the use of facial make up products (foundation and blush). The upper-bounding estimate of combined use of multiple products resulted in an upper-bounding applied dose of 4.2 mg/kg-bw per day. For personal care products that are used less frequently, such as body makeup, hair dye, bath preparation and manicure preparation products, exposure was estimated per use of product for each use. The highest exposure estimate resulted from the use of body makeup, where the upper-bounding exposure estimate was 13.3 mg/kg-bw per use.

Table 8. Upper-bounding applied dose of Solvent Red 48 from use of personal care products (chronic and acute exposures)

Product		**Concentration range (%)**	**Oral**	**Dermal**
Chronic exposure (mg/kg-bw/day)				
Lipstick		≤ 30	≤ 0.20	-
Skin moisturizer		≤ 0.1	-	≤ 2.00
Face makeup	Foundation	≤ 10	-	≤ 1.33
	Blush	≤ 30	-	≤ 0.67
Eye makeup	Eye mascara	10–30	-	0.03–0.13
	Eye shadow	≤ 10	-	≤ 0.03
	Eyeliner	≤ 10	-	≤ 0.01
Skin cleanser		≤ 0.3	-	≤ 0.03
Fragrance		≤ 0.1	-	≤ 0.03
Total chronic			**≤ 0.20**	**≤ 4.23**
Acute exposure (mg/kg-bw) per use				
Body makeup		0.3–30	-	0.20–13.3
Hair dye		≤ 3	-	≤ 4.00

Product	Concentration range (%)	Oral	Dermal
Nail white pencil	10–30	-	0.03–1.33
Bath preparation	≤ 0.3	-	≤ 0.13
Nail polish	≤ 10	-	≤ 0.07
Nail polish remover	≤ 0.3	-	≤ 0.01

"-" not applicable

As the CNS database is not directly linked to CAS RNs, uncertainty exists that the notified substance may not correspond to the CAS RN of interest even though it corresponds to the substance name "Solvent Red 48", Considering the complex chemistry of this substance as a colorant, all products associated with the following names are considered relevant to this assessment: Solvent Red 48, C.I. 45410, D&C Red No.27, D&C Red No.28, Red 27, and Red 28 (Appendix 3).

As the weight fractions of Solvent Red 48 used to derive exposure estimates are based on the maximum concentration in the reported range of concentrations in the CNS database, confidence is high that these exposure estimates are conservative.

Solvent Red 48 is not volatile, hence inhalation exposures to this substance are not expected from use of personal care products.

Health Effects Assessment

The following hazard summary for Solvent Red 48 (acid) and Phloxine B (sodium salt) is based primarily on data cited in risk assessments conducted by the U.S. FDA[2] (Lipman 1995; US FDA 1982a, 1982b) and the European Scientific Committee on Cosmetic Products and Non-food products intended for Consumers (SCCNFP 2004).[3] Additional studies not cited in these assessments have also been considered where relevant. A summary of the hazard data for Solvent Red 48 and Phloxine B is presented below, and with more detail in Appendices 4 and 5.

The U.S. FDA evaluated the safety of Solvent Red 48 (D&C Red No.27) and Phloxine B (D&C Red No.28) as part of a petition for their approved use as colour additives in cosmetics and drugs (Lipman 1995; US FDA 1982a, 1982b). Phloxine B is the disodium salt of Solvent Red 48 and therefore Solvent Red 48 and Phloxine B are a conjugate acid-base pair. The transition from Solvent Red 48 to Phloxine B occurs between pH 3.4 and pH 5.0 (CRC 1986). Hence, it can be assumed that Phloxine B will be the dominant species under physiological pH (approximately 7.4). Furthermore, Solvent Red 48 and Phloxine B were considered to be "toxicologically equivalent" by the U.S. FDA and the

[2] The evaluation was for D&C Red No.27 and D&C Red No.28. Many of the original studies submitted to the U.S. FDA were not evaluated for this assessment; rather, the study details and conclusions of the U.S. FDA were cited from a literature review by the U.S. FDA (Lipman 1995) and/or associated entries for these substances in the U.S. Federal Register (US FDA 1973, 1982a, 1982b).
[3] The evaluation was for "CI Acid Red 92." Many of the original studies cited in the Scientific Committee on Cosmetics and Non-Food Products (SCCNFP) report (2004) were not evaluated for this assessment; rather, the study details and conclusions are summarized as reported by the SCCNFP.

group conclusion on safety was based on studies from both chemical forms (US FDA 1982a). Therefore, data for both substances are considered collectively for this screening assessment.

Several studies investigating the potential carcinogenicity of Solvent Red 48 and Phloxine B were identified. Chronic studies on Solvent Red 48 submitted to the FDA as part of the petition for colorant use included multiple oral studies in mice (Litton Bionetics 1981a; Procter and Gamble 1992), rats (Litton Bionetics 1981b; Industrial Bio-Test Labs 1965a), and in beagles (Industrial Bio-Test Labs 1965b) as well as dermal studies in rabbits (Leberco Laboratories 1968) and mice (Hazleton Laboratories 1969; Carson 1984). Due to limitations of some of the older studies submitted, the FDA considered the pivotal data for the carcinogenicity conclusion to be based on the more recent chronic studies in the mouse (Litton Bionetics 1981a; Procter and Gamble 1992) and rat (Litton Bionetics 1981b), which were conducted in accordance with the updated FDA standards (Lipman 1995). Based on these newer chronic studies, the U.S. FDA concluded that D&C Red No. 27 & 28 (Solvent Red 48 and its sodium salt) were not carcinogenic in rats or mice following lifetime dietary exposure, and that the substances were safe for use as colorants in drugs and cosmetics (US FDA 1982b; Lipman 1995). For Phloxine B, a chronic study was identified for mice, which were exposed via the diet at concentrations up to 0.4% (approximately 520 mg/kg-bw per day) for 90 weeks. While an increased incidence of pituitary tumours were reported in female mice, the increases were not dose-dependent. The study authors concluded that Phloxine B was not carcinogenic in mice based on this study (Ito et al. 1994). A review of the Ito et al. study by the Scientific Committee on Cosmetics and Non-Food Products (SCCNFP) did not reach a conclusion on carcinogenicity, although they considered the study to be not adequately reported (SCCNFP 2004).

Solvent Red 48 induced mutations in *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA104, TA1535 and TA1537 with and without metabolic activation (NTP 2002), while inconclusive results were obtained from DNA repair assays in *Bacillus subtilis* strains H17 and M45 (GENETOX 1991). No *in vivo* genotoxicity studies were identified in the literature for Solvent Red 48. Phloxine B was not clastogenic *in vivo* (mouse bone marrow) and was also negative for gene mutations in mouse lymphoma cells and *Salmonella typhimurium* both with and without metabolic activation (SCCNFP 2004; Maus et al. 1981). While positive results for reverse mutations in *Escherichia coli* strains B/r WP2 were reported (Kada et al. 1972), negative results were obtained under similar experimental conditions (Maus et al. 1981).

A summary of the lowest effect levels for non-cancer effects following repeated-dose exposures to Solvent Red 48 and Phloxine B are given below.

For Solvent Red 48, the lowest identified oral LOELs (lowest-observed-effect level) from the available repeat-dose studies ranged from 500 mg/kg-bw per day for a chronic study in rats[4] (NOEL [no-observed-effect level] of 125mg/kg-bw per day, Litton Bionetics

[4] Specific effects associated with the NOEL/LOEL for this study were not provided in the secondary source (Litton Bionetics 1981b, as cited in Lipman 1995), and the original unpublished study was not available. See the Appendix for details.

1981b) to 1000 mg/kg-bw per day for a subchronic rat study based on treatment-related decreases in growth (NOEL = 500mg/kg-bw per day, Hansen et al. 1958, abstract only). No other effects were identified in the available literature for Solvent Red 48. The various earlier studies submitted to the U.S. FDA did not appear to demonstrate any exposure-related effects for developmental or reproductive toxicity by diet and gavage respectively (cited in NTP 2000). In addition, no exposure-related effects were reported for a series of dermal studies submitted to the U.S. FDA, including short-term and subchronic exposure in rabbits (5x/week at 0.1 and 1%, Leberco Laboratories 1968) and chronic exposure in mice (2x/week at 1%, Carson et al. 1984). Although the microscopic examination for the chronic study focused primarily on skin and only grossly abnormal tissues, the lack of effects in these studies suggests a low hazard via the dermal route. Due to study limitations, these older studies were not considered by the U.S. FDA to meet current standards for toxicological testing (US FDA 1982b). No irritation or sensitization studies were identified in the literature for Solvent Red 48.

The FDA established an acceptable daily intake (ADI) for D&C Red No. 27 and No. 28 at 1.25 mg/kg-bw per day on the basis of an oral NOEL of 125 mg/kg-bw per day derived from the chronic rat study described above (Lipman 1995).[5]

For Phloxine B, developmental effects and adverse effects in the gastrointestinal tract (GIT), liver and clinical chemistry have been reported in rodents following repeat-dose oral exposures; no dermal or inhalation studies were identified. The lowest oral LOEL for short-term exposures was 250 mg/kg-bw per day (NOEL of 50mg/kg-bw per day) based on a treatment-related decrease in basophil count and an increase in platelet count for female rats exposed for 4 weeks via drinking water (SCCNFP 2004). The lowest LOEL for subchronic 13-week drinking water exposure in rats was also 250 mg/kg-bw per day (NOEL of 50mg/kg-bw per day) based on multiple effects (myosis, decreased locomotor activity, increased absolute eosinophil count, altered clinical chemistry, impaired urine-concentrating ability, increased urine pH) (SCCNFP 2004). In addition, stomach irritation and reddish feces were a common finding in the above studies, and it is therefore plausible that the hematological, biochemistry and urinalysis effects noted above may have been related to stomach irritation. Therefore, the toxicological relevance of these findings was not clear, so the NOELs/LOELs above represent effect levels only. In the only chronic study for Phloxine B, exposure to mice for 90 weeks via the diet (0, 0.1, or 0.4%) resulted in a dose-dependent and statistically significant increase in mean liver weights at both doses (approximately 130 and 520 mg/kg-bw per day, respectively). Increased body weights and fat deposition for males and females of both dose groups indicated an increased palatability of the Phloxine B–treated feed (Ito et al. 1994), which adds uncertainty to interpretation of the increased liver weights. An oral study also reported developmental effects at the lowest tested dose of 1% Phloxine B in the diet (approximately 1300 mg/kg-bw per day) based on a dose-related incidence of splitting of the cervical vertebral arches in rat fetuses exposed *in utero* (Seno et al. 1984). Another study of pregnant rats exposed during gestation at concentrations up to 3% in the diet did not show apparent developmental effects from exposure, although the study authors

[5] The ADI derived by the U.S. FDA was based on studies for D&C Red No. 27 (acid form, Solvent Red 48) and did not consider studies on the Na salt reviewed by the SCCNFP (2004).

considered systemic exposure to have occurred by detection of dye fluorescence in tissues of the dams and fetuses (Nakaura et al. 1975). Other hazard data for Solvent Red 48 and Phloxine B are summarized in Appendices 4 and 5.

Although clear signs of systemic toxicity were not evident, the collective toxicity data set suggests that the ionized form (Phloxine B) may be more biologically active than the acid form (Solvent Red 48), potentially due to the molecular conversion between the two species. As indicated previously, the quinonoid ionized form would be dominant at neutral pH and above, while the acid form would be only be present at very low pH. The acid has low water solubility and would tend to precipitate out as observed for eosin, an analogue xanthene dye (Levillain and Fompeydie 1985). While the acid may be reported as the dosage form for a given animal study (i.e., all studies submitted to the U.S. FDA were reportedly for D&C Red No. 27, the acid form), conversion to the more soluble quinonoid ion would tend to occur in the basic aqueous environment of the lower gastrointestinal tract (GIT). Since the acid lactone is colourless, the presence of red colouration of the feces and in the GIT at necropsy, in studies reportedly dosing as the acid form, further support that the conversion does take place from lactonic acid to quinonoid acid, then to ionized quinonoid. However, since the rate of this conversion is not known, it is conceivable that there may be insufficient time for complete conversion to the soluble ion in the GIT. Thus, it is possible that studies dosing as the acid form may have a relatively lower soluble fraction compared to the salt which would be completely dissociated and soluble before entering the GIT. For illustration, the lowest chronic oral LOELs for dietary exposures to Solvent Red 48 (acid) and Phloxine B (salt) were 500 and ≤ 130-250 mg/kg-bw per day, respectively. However details on specific dosage forms for many of the reported studies are insufficient to make any definitive conclusions on the differential hazards for the two molecular species..

Regarding the metabolism and excretion patterns of Solvent Red 48, it was reported that the acid form of Solvent Red 48 had a 100% (approximate) recovery rate from the excreta following oral exposures in rats, indicating either very limited oral absorption and/or absorption followed by near complete hepatic clearance in the bile (Webb et al. 1962). The very limited, low oral absorption of this substance is supported by a more recent study for the salt form, which suggested an oral bioavailability of 0.35–1.5% following a single bolus oral dose (50 and 500mg/kg-bw respectively), with fecal excretion accounting for 98% of the administered dose (Sweet et al. 2004). The study authors attributed the higher absorption of 1.5% to GIT irritation observed for the higher bolus dose of 500mg/kg-bw. Therefore, the absorption rate of 0.35% without observed GIT irritation at the bolus dose of 50mg/kg-bw is considered more reflective of absorption from dietary exposure to Solvent Red 48. Furthermore, since an estimated 0.14% of the 0.35% absorbed was collected in the bile 3 hrs post-dosing, this suggests that rapid hepatic excretion would further limit systemic exposure to the already limited absorbed fraction. Dermal absorption was considered negligible in the *in vitro* percutaneous penetration studies of the acid and sodium salt (as D&C Red No. 27 and 28) on human skin, in which neither substance was detected with the detection limit 5ng/ml in the receptor fluid up to 24 hrs post-dosing (2010 personal communication from US NTP to Existing Substances Risk Assessment Bureau, Health Canada; unreferenced). The

lack of effects observed in dermal repeat-dose studies in rabbits and mice also suggest limited potential for dermal absorption. Furthermore, limited dermal penetration was also observed for the analogue Rose Bengal (iodine instead of bromine substitution) following single exposures in mice and repeated dosing in rabbits (Wachter et al. 2003). Therefore, on the basis of available information, it is considered that absorption of both Solvent Red 48 (acid) and Phloxine B (sodium salt) is likely very low following oral and dermal exposure.

The potential phototoxicity of Solvent Red 48 and Phloxine B has also been described (NTP 2000). Photo-excitation of these dyes can result in the formation of free radicals (type I photodynamic mechanism) or singlet oxygen (type II photodynamic mechanism) (NTP 2000). The phototoxic effects exhibited by Phloxine B have resulted in its investigation for use in the United States as a potential photoactive insecticide (Heitz 1997). Based on this potential health effect, an investigation of potential for dermal absorption was initiated by the U.S. FDA/NTP (see above). However, results of these studies indicate limited potential for dermal absorption (2010 personal communication from U.S. NTP to Existing Substances Risk Assessment Bureau, Health Canada; unreferenced). Therefore, phototoxicity is not considered a critical health effect in this assessment.

Although hazard data for Solvent Red 48 and Phloxine B were identified for multiple endpoints, including acute and repeated-dose toxicity, reproductive and developmental toxicity, carcinogenicity and genotoxicity, confidence in the data is somewhat lowered due to the limited level of detail reported and limited access to the primary references. In addition, no epidemiological studies were available in the literature for these substances.

Characterization of Risk to Human Health

In multiple chronic studies in mice and rats available for Solvent Red 48 evaluated by the U.S. FDA, no evidence for carcinogenicity was observed. Although one positive mutagenicity study in bacteria was reported for Solvent Red 48, there were multiple negative genotoxicity studies *in vivo* and *in vitro* for Phloxine B. Therefore based on collective data for both molecular forms, Solvent Red 48 is not considered to be genotoxic.

The principal source of exposure to Solvent Red 48 is considered to be the use of personal care products containing the substance. The upper-bounding chronic oral exposure from lipstick is estimated to be 0.2 mg/kg-bw per day, which is well below the oral-based ADI (1.25 mg/kg-bw per day) established by the U.S. FDA.

While the upper-bounding aggregate chronic dermal exposure to Solvent Red 48 from the use of multiple products (skin moisturizer, eye and facial makeup, skin cleanser, and fragrance) on a same day was estimated to be 4.2 mg/kg-bw per day of applied dose, most of the contribution is due to exposure to skin moisturizer and facial makeup products. Occasional use of other products can result in higher acute dermal exposures.

Given that the health effects database for Solvent Red 48 does not indicate a high hazard; that limited toxicity studies by the dermal route did not demonstrate systemic effects; and that potential for dermal absorption is very limited, exposures via the dermal route were not considered to be of concern for human health.

Uncertainties in Evaluation of Risk to Human Health

There are uncertainties in the hazard database due to the numerous studies cited by the U.S. FDA and SCCNFP, many of which were not published in the open literature or were only available in abstract form. For example, the critical effects associated with the lowest chronic oral NOEL and derived ADI for D&C Red No. 27 (Solvent Red 48) were not indicated in the limited secondary reviews (Litton Bionetics 1981b, cited in Lipman 1995 and NTP 2000). While none of the unpublished studies cited here were critically evaluated by Health Canada for the purposes of this assessment, the study results were extracted from reputable secondary reviews (i.e., U.S. FDA and SCCNFP).

It should also be noted that while the U.S. FDA evaluation emphasized the toxicological equivalency of both D&C Red No. 27 (Solvent Red 48, acid) and D&C Red No. 28 (Phloxine B, salt), studies on the salt/ionized form reviewed by the SCCNFP (2004) were not specifically considered in the U.S. FDA evaluation.

There is also uncertainty regarding the potential phototoxicity of Solvent Red 48 and Phloxine B (NTP 2000). However, the limited skin penetration from *in vitro* percutaneous absorption studies on these substances (2010 personal communication from US FDA to Existing Substances Risk Assessment Bureau, Health Canada; unreferenced) suggests a low potential risk for this effect.

Uncertainty is high with respect to the extent of the exposure of the general population to Solvent Red 48 from personal care products. However, as all information associated with Solvent Red 48 in the CNS database was considered to derive exposure estimates for this substance from the use of personal care products, the confidence is high that the derived exposure estimates are very conservative. In addition, maximum concentration in the reported concentration range was used to derive the exposure estimates. Therefore, additional information on actual concentration of Solvent Red 48 in cosmetic products would further refine the exposure characterization.

Some uncertainty is associated with the potential exposure to Solvent Red 48 from pharmaceutical drugs, natural health products and veterinary drugs, which would contribute to oral exposure.

Conclusion

Based on the information presented in this final screening assessment, it is concluded that Solvent Red 48 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.

Additionally, based on the information available, it is proposed that Solvent Red 48 is not a substance 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 48 does not meet any of the criteria set out in section 64 of CEPA 1999. Additionally, Solvent Red 48 meets the criteria for persistence in water, soil and sediment, but does not meet the criteria for bioaccumulation potential as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

This substance will be considered for inclusion in the Domestic Substances List inventory update initiative. In addition, and where relevant, research and monitoring will support verification of assumptions used during the screening assessment.

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Appendix 1 – Robust Study Summary

No	Item	Weight	Yes/No	Specify
1	Reference: The acute and chronic toxicity of two xanthene dyes, fluorescein and sodium sald and phloxine B, to *Daphnia pulex.* Walthall and Stark 1999. Environmental Pollution. Vol. 104, pp. 207-215			
2	Substance identity: CAS RN	n/a	Y	18472-87-2
3	Substance identity: chemical name(s)	n/a	Y	Phloxine B
4	Chemical composition of the substance	2	N	
5	Chemical purity	1	N	
6	Persistence/stability of test substance in aquatic solution reported?	1	Y	Mortality half-life (MT50) for test organism under test conditions was relatively quick due to photodegradation under fluorescent lights
	Method			
7	Reference	1	Y	
8	OECD, EU, national, or other standard method?	3	N	
9	Justification of the method/protocol if not a standard method was used	2		n/a
10	GLP (Good Laboratory Practice)	3	N	
	Test organism			
11	Organism identity: name	n/a	Y	*Daphnia pulex*
12	Latin or both Latin & common names reported?	1	Y	
13	Life cycle age / stage of test organism	1	Y	
14	Length and/or weight	1		n/a
15	Sex	1		n/a
16	Number of organisms per replicate	1	Y	5 or 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	both acute and chronic
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	acute exposure: 48 h; chronic exposure: 10 days
23	Negative or positive controls (specify)	1	Y	negative
24	Number of replicates (including controls)	1	Y	4
25	Nominal concentrations reported?	1	Y	5
26	Measured concentrations reported?	3	N	
27	Food type and feeding periods during the long-term tests	1	Y	
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 the metal toxicity - pH, DOC/TOC, water hardness, temperature)	3	Y	
30	Photoperiod and light intensity	1	Y	16h:8h light dark
31	Stock and test solution preparation	1	Y	
32	Was solubilizer/emulsifier used, if the chemical was poorly soluble or unstable?	1		n/a
33	If solubilizer/emulsifier was used, was its concentration reported?	1		n/a
34	If solubilizer/emulsifier was used, was its ecotoxicity reported?	1		n/a
35	Monitoring intervals (including observations and water quality parameters) reported?	1	N	
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 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	7.4-7.8
42	Was temperature of the test water within the range typical for the Canadian environment (5 to 27°C)?	1	Y	25±0.1 C
43	Was toxicity value below the chemical's water solubility?	3	Y	
	Results			
44	Toxicity values (specify endpoint and value)	n/a	n/a	48-h LC50 (95% fiducial limits) of phloxine B =0.423 (0.376-0.477) mg/L
45	Other endpoints reported - e.g. BCF/BAF, LOEC/NOEC (specify)?	n/a	Y	48-h LC90 of phloxine B = 0.952 (0.795-1.23); 10-day chronic effect on fecundity starting at 1 mg/L and greater
46	Other adverse effects (e.g. carcinogenicity, mutagenicity) reported?	n/a	N	
47	***Score*** : ... %			*66.7*
48	***EC Reliability code*** :			*2*
49	***Reliability category (high, satisfactory, low)*** :			*Satisfactory Confidence*
50	***Comments***			

Appendix 2 – PBT Model Inputs Summary Table

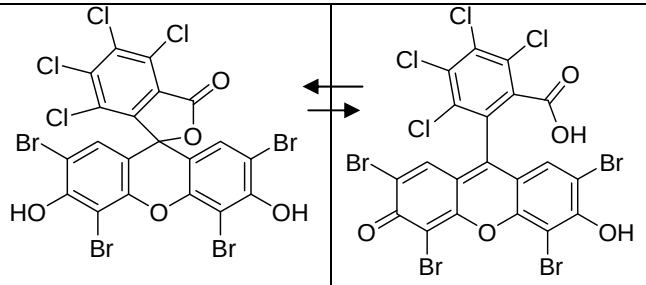
	Phys-Chem/Fate	Fate	PBT Profiling	Ecotoxicity
Model Input Parameters	EPIWIN Suite (all models, including: AOPWIN, KOCWIN, BCFWIN, BIOWIN and ECOSAR)	Arnot-Gobas BCF/BAF Model	Canadian-POPs (including: Catabol, BCF Mitigating Factors Model, OASIS Toxicity Model)	Artificial Intelligence Expert System (AIES/ TOPKAT/ ASTER)
SMILES Code	O=C1C(Br)=C2Oc3c(Br)c(O)c(Br)cc3C(c4c(Cl)c(Cl)c(Cl)c(Cl)c4C(=O)O)=C2C=C1Br	O=C1C(Br)=C2Oc3c(Br)c(O)c(Br)cc3C(c4c(Cl)c(Cl)c(Cl)c(Cl)c4C(=O)O)=C2C=C1Br	O=C1C(Br)=C2Oc3c(Br)c(O)c(Br)cc3C(c4c(Cl)c(Cl)c(Cl)c(Cl)c4C(=O)O)=C2C=C1Br	O=C1C(Br)=C2Oc3c(Br)c(O)c(Br)cc3C(c4c(Cl)c(Cl)c(Cl)c(Cl)c4C(=O)O)=C2C=C1Br
Molecular weight (g/mol)	785.68	785.68	785.68	785.68
Melting point (ºC)				
Boiling point (ºC)				
Data temperature (ºC)				
Density (kg/m^3)				
Vapour pressure (Pa)				
Henry's Law constant (Pa·m^3/mol)				
Log K_{aw} (Air-water partition coefficient) (dimensionless)				
Log K_{ow} (Octanol-water partition coefficient) (dimensionless)	2.30[1] for K_{oc} and LC_{50}	2.30[1]	2.30[1]	
Log K_{oc} (Organic carbon-water partition coefficient – L/kg)				
Water solubility (mg/L)				

Log K_{oa} (Octanol-air partition coefficient) (dimensionless)				

[1]Modelled using experimental value adjustment (EVA) with Phloxine B empirical Log K_{ow} = -0.21.

Appendix 3 – Summary of Structural Association of CAS RNs and Common Names for Solvent Red 48 and Phloxine B

(Amat-Guerri et al. 1990b; PCPC 2010;US FDA 1982c; Levillain and Fompeydie 1985; Zuckerman 1974)

Name used in screening assessment	**Solvent Red 48**		**Phloxine B**	
U.S. FDA name	D&C Red No. 27		D&C Red No.28	
CI name	CI Solvent Red 48		CI Acid Red 92	
CI number	CI 45410, CI 45410:A or CI 45410:1		CI 45410	
CAS RN	13473-26-2	2134-15-8	18472-87-2	4618-23-9
Tautomer	lactone	quinonoid	lactone	quinonoid
Ionization	acid	acid	di-anion of sodium salt	di-anion of sodium salt
Approximate pH favouring formation	< 2 (lactone is insoluble in aqueous solutions)		> 5 (Na ions dissociate in aqueous solutions to give di-anion)	
Molecular structure				

Appendix 4 a) – Upper-bounding Oral Exposure Estimates to Solvent Red 48 in Lipsticks Using ConsExpo 4.1 (ConsExpo 2006)

Consumer product scenarios	Assumptions[1]	Estimated exposure
Chronic Exposures **(per day)**		
Lipstick	Concentration of Solvent Red 48 ≤ 30% [2] Exposure frequency: 1460×/year (RIVM 2006) **Oral route** Exposure type: Direct intake (ConsExpo 2006) Amount of product ingested: 0.01 g (RIVM 2006)	Chronic applied dose[3] ≤ 0.20 mg/kg-bw per day

[1] The following assumptions were applied to all scenarios:
- body weight of 70.9 kg for an adult;
- exposure type of "direct dermal contact" for instant application (ConsExpo 2006).

[2] Based on concentrations as reported on the Cosmetics Notification System (CNS 2009).

[3] Chronic oral dose calculated through amortization over a year.

Appendix 4 b) – Upper-bounding Dermal Exposure Estimates to Solvent Red 48 in Personal Care Products Using ConsExpo 4.1 (ConsExpo 2006)

Consumer product scenarios	Assumptions[1]	Estimated exposure
Chronic Exposures **(per day)**		
Blush	Concentration of Solvent Red 48 ≤ 30% [2] Exposure frequency: 365×/year (RIVM 2006) Exposed area: 160 cm^2 (Health Canada 1995) Amount of product applied: 0.23 g [4]	Chronic applied dose[3] ≤ 0.667 mg/kg-bw per day
Eye mascara	Concentration of Solvent Red 48 = 10–30% [2] Exposure frequency: 365×/year (RIVM 2006) Exposed area: 1.6 cm^2 (RIVM 2006) Amount of product applied: 0.025 g (RIVM 2006)	Chronic external applied dose[3] = 0.033–0.133 mg/kg-bw per day
Eye shadow	Concentration of Solvent Red 48 ≤ 10% [2] Exposure frequency: 730×/year (RIVM 2006) Exposed area: 24 cm^2 (RIVM 2006) Amount of product applied: 0.01 g (RIVM 2006)	Chronic applied dose[3] ≤ 0.027 mg/kg-bw per day
Eyeliner	Concentration of Solvent Red 48 ≤ 10% [2] Exposure frequency: 365×/year (RIVM 2006) Exposed area: 3.2 cm^2 (RIVM 2006) Amount of product applied: 0.005 g (RIVM 2006)	Chronic applied dose[3] ≤ 0.007 mg/kg-bw per day
Blush	Concentration of Solvent Red 48 ≤ 30% [2] Exposure frequency: 365×/year (RIVM 2006) Exposed area: 160 cm^2 (Health Canada 1995) Amount of product applied: 0.23 g [4]	Chronic dose[3] ≤ 0.667 mg/kg-bw per day
Foundation	Concentration of Solvent Red 48 ≤ 10% [2] Exposure frequency: 365×/year (RIVM 2006) Exposed area: 638 cm^2 (Health Canada 1995) Amount of product applied: 0.8 g	Chronic applied dose[3] ≤ 1.33 mg/kg-bw per day
Fragrance	Concentration of Solvent Red 48 ≤ 0.1% [2] Exposure frequency: 1095×/year (RIVM 2006)	Chronic applied dose[3] ≤ 0.027 mg/kg-

Consumer product scenarios	**Assumptions**[1]	**Estimated exposure**
	Exposed area: 200 cm^2 (ConsExpo 2006) Amount of product applied: 0.64 g (ConsExpo 2006)	bw per day
Skin cleanser	Concentration of Solvent Red 48 ≤ 0.3% [2] Exposure frequency: 329×/year (RIVM 2006) Exposed area: 18200 cm^2 (Health Canada 1995) Amount of product applied: 26.1 g Retention Factor: 10%	Chronic applied dose[3] ≤ 0.033 mg/kg-bw per day
Skin moisturizer	Concentration of Solvent Red 48 ≤ 0.1% [2] Exposure frequency: 730×/year (RIVM 2006) Exposed area: 16925 cm^2 (Health Canada 1995) Amount of product applied: 8 g [4]	Chronic applied dose ≤ 2.00 mg/kg-bw per day
***Acute Exposures* (per use)**		
Bath preparation (e.g., salts, tablets)	Concentration of Solvent Red 48 ≤ 0.3% [2] Exposed area: 16925 cm^2 (Health Canada 1995) Amount of product applied: 16925 g	Per-use external applied dose ≤ 0.133 mg/kg-bw per event
Body makeup (e.g., shimmer powder, shimmer lotion, makeup stick)	Concentration of Solvent Red 48 = 0.3–30% [2] Exposed area: 8370 cm^2 (Health Canada 1995) Amount of product applied: 3.5–4 g (depending on product)[4]	Per-use external applied dose = 0.2–13.0 mg/kg-bw per event
Hair dye	Concentration of Solvent Red 48 ≤ 3% [2] Exposed area: 638 cm^2 (Health Canada 1995) Amount of product applied: 100 g [4] Retention Factor: 10%	Per-use external applied dose ≤ 4.00 mg/kg-bw per event
Nail polish	Concentration of Solvent Red 48 ≤ 10% [2] Exposed area: 4 cm^2 (RIVM 2006) Amount of product applied: 0.05 g (RIVM 2006)	Per-use external applied dose ≤ 0.067 mg/kg-bw per event
Nail white pencil	Concentration of Solvent Red 48 = 10–30% [2] Exposed area: 2 cm^2 (RIVM 2006) Amount of product applied: 0.025 g (RIVM 2006)	Per-use external applied dose = 0.033–0.133 mg/kg-bw per event
Nail polish remover	Concentration of Solvent Red 48 ≤ 0.3% [2] Exposed area: 11 cm^2 (RIVM 2006) Amount of product applied: 0.2 g (RIVM 2006)	Per-use external applied dose ≤ 0.007 mg/kg-bw per event

[1] The following assumptions were applied to all scenarios:
- body weight of 70.9 kg for an adult;
- uptake fraction of 1.5% for both dermal and orgal absorption;
- exposure type of "direct dermal contact" for instant application (ConsExpo 2006).

[2] Based on concentrations as reported on the Cosmetics Notification System (CNS 2009).

[3] Chronic applied dose calculated through amortization over a year.

[4] Calculated by multiplying the product amounts as stated in RIVM 2006 with the ratio of the surface area of the affected body surface as reported in Health Canada 1995 with that of RIVM 2006.

Appendix 5 – Summary of Health Effects Information for Solvent Red 48 (Acid)

Endpoint	**Lowest effect levels/Results** (see notes 1–5 at end of this table)
Laboratory animals and *in vitro* assays	
Acute toxicity	**Lowest oral LD_{50}** = 8400 mg/kg-bw in male Sprague Dawley rats (Industrial Bio-Test Labs 1962a). Additional studies: Industrial Bio-Test Labs (1962b). No inhalation or dermal or LD_{50} values were identified.
Short-term repeated-dose toxicity	*Dermal* Rabbits (unspecified strain, female, 6 animals per group) were administered Solvent Red 48 in hydrophilic or U.S.P. white ointment topically at concentrations of 0.1% or 1.0%, daily, 5 days per week for 28 days (applied volumes were not provided in secondary reference, so an applied dose in mg/kg-bw per day could not be derived). Control groups received white ointment only. For each treatment duration, 3 rabbits received treatment on intact skin and 3 rabbits were treated on abraded skin. Body weights and the time for abraded skin to heal were not affected by the administration of Solvent Red 48. No exposure-related gross and histopathological changes in the internal organs were reported. No LOEL or NOEL for this study could be estimated due to lack of information (limited details are provided due to the study being available only as an abstract) (Leberco Laboratories 1968). No other dermal studies were identified in the literature. No short-term oral or inhalation toxicity studies were identified in the literature.
Subchronic toxicity	*Oral* **Lowest oral LOEL** = 1000 mg/kg-bw. Rats (albino, unspecified strain, weanling, 10 animals per sex per group) were exposed to Solvent Red 48 via the diet at concentrations of 0, 0.25, 0.5, 1 or 2% (approximately 0, 125, 250, 500 and 1000 mg/kg-bw per day; see note 2 at end of Appendix for dose conversion) for 18 weeks. At the 2% level, rats showed retarded growth (limited details are provided, as the study was available only as an abstract) (Hansen et al. 1958). No other oral studies were identified in the literature. *Dermal* Rabbits (unspecified strain, female, 6 animals per group) were administered Solvent Red 48, in hydrophilic or U.S.P. white ointment topically at concentrations of 0.1% or 1.0% daily, 5 days per week for 91 days (applied volumes were not provided in secondary reference, so an applied dose in mg/kg-bw per day could not be derived). Control groups received white ointment only. For each treatment duration, 3 rabbits received treatment on intact skin and 3 rabbits were treated on abraded skin. Body weights and the time for abraded skin to heal were not affected by the administration of Solvent Red 48. No exposure-related gross and histopathological changes in the internal organs were reported. No LOEL or NOEL for this study could be estimated due to lack of information (limited details are provided due to the study being available only as an abstract) (Leberco Laboratories 1968). No other dermal studies were identified in the literature. No subchronic inhalation toxicity studies were identified in the literature.
Chronic toxicity/	*Oral:*

Endpoint	**Lowest effect levels/Results** (see notes 1–5 at end of this table)
carcinogenicity	**LOEL** = 500mg/kg-bw per day (NOEL = 125 mg/kg-bw per day) F_0 Rats (CD, 60 animals per sex per group) were exposed to Solvent Red 48 via diet at concentrations of 0.25, 1.00 and 2.00% (approximately 125, 500 or 1000 mg/kg-bw per day; see note 2 at end of Appendix for dose conversion) for eight weeks prior to mating and through mating and gestation, and lactation and weaning of litters. When weanlings (F_1) were five weeks old, they were separated from the dams and cycled through the same treatment regimen as the F_0 generation (approximately 250, 1000 or 2000 mg/kg-bw per day; see note 2 at end of Appendix for dose conversion). The FDA determined the NOEL for this study to be 125 mg/kg-bw per day (Lipman 1995; Litton Bionetics 1981b)[6] (specific effects observed at the LOEL were not reported). This NOEL was used for the calculation of an ADI = 1.25mg/kg-bw per day by applying a safety factor of 100 (Lipman 1995; Litton Bionetics 1981b). Mice (CD-1, 60 animals per sex per group) were exposed to Solvent Red 48 via diet at concentrations of 1250, 5000 and 10 000 ppm (approximately 160, 650 or 1300 mg/kg-bw per day; see note 3 at end of Appendix for dose conversion) for 2 years. No exposure-related effects were noted other than pink colouration of the experimental animals. No exposure-related changes were noted in survival rates, gross observations, body weights and food intake. In addition, no gross or histopathological changes were observed. No significant increases in the incidence of tumours were reported. Pink colouration of some of the internal organs were noted upon autopsy, suggesting there was some absorption and systemic exposure to Solvent Red 48 at these doses. The investigators concluded that Solvent Red 48 is not carcinogenic in mice (Procter and Gamble 1992, also cited as Litton Bionetics 1981a[7]). Rats (Sprague Dawley, 25 animals per sex per group) were exposed to Solvent Red 48 via diet at concentrations of 0.015, 0.25 and 1.0 % for 2 years (approximately 7.5, 125 or 500 mg/kg-bw per day; see note 2 at end of Appendix for dose conversion). No significant treatment-related effects were noted for body weight, organ weight, hematologic determinations and urinalysis (Industrial Bio-Test Labs 1965a). Dogs (beagles, 3 animals per sex per group) were exposed to Solvent Red 48 via diet at concentrations of 0.015, 0.25 and 1.0% (approximately 4.5, 75 or 300 mg/kg-bw per day; see note 4 at end of Appendix for dose conversion) for 720 days. No significant treatment-related effects were noted for blood chemistries, urinalysis determinations, behaviour, body weight, food consumption, or histopathology (Industrial Bio-Test Labs 1965b). *Dermal:* Mice (ICR, 50 animals per sex per dose) were exposed to Solvent Red 48 topically at 1 mg in 0.1 ml of distilled water (1% aqueous suspension) or vehicle alone (water) on clipped dorsal skin (6 cm^2) twice per week for 18 months (dye treatment group equivalent to approximately 9.5 mg/kg-bw per day; see note 5 at end of Appendix for dose conversion). Dead, moribund and

[6] Although the full FDA evaluation of this study was not available, it is assumed that Litton Bionetics (1981b) corresponds to the "new" chronic rat study cited in Lipman (1995) which was used for derivation of an ADI = 1.25 mg/kg-bw per day based on a NOEL = 125 mg/kg-bw per day (0.25% in diet). Although Lipman (1995) does not specifically mention the LOEL or effects observed for this study, it is assumed that the LOEL for this study is 500 mg/kg-bw per day based on the next higher dietary dose of 1%.

[7] Although the original study of Litton Bionetics (1981a) was not available and only limited information is provided in the secondary reference (NTP 2000), it is believed that this is the same data reported in Procter and Gamble (1992). However, the NTP (2000) reports the highest tested dose to be 5% (50 000 ppm) and duration of 18 months for Litton Bionetics (1981a), whereas Procter and Gamble (1992) reports the highest dose to be 1% (10 000 ppm) and a duration of 2 yrs.

Endpoint	**Lowest effect levels/Results** (see notes 1–5 at end of this table)
	surviving animals to 18 months were necropsied at the end of the study. Histopathology was performed on skin and only on those other organs/tissues that appeared grossly abnormal. From this analysis the authors concluded that there were no exposure-related changes in survival and no increased incidence in tumours or skin lesions when compared to the vehicle control. (Since the toxicological endpoints were limited to a histopathology examination on the skin and only grossly abnormal tissues, it is not considered that a NOEL for systemic effects was demonstrated for this study.) (Hazleton Laboratories 1969, Carson 1984[8]). No other chronic toxicity/carcinogenicity studies were identified in the available literature.
Reproductive toxicity	*Oral* **Highest NOEL** = 1000 mg/kg-bw per day (no reproductive effects observed at highest tested dose). (Limited details provided, since only the abstract was available for this study.) Rats (unspecified strain, unspecified number of animals per sex per group) were exposed to Solvent Red 48 via diet at concentrations of 1x, 10x, 30x, and 100x the Acceptable Daily Intake level or the projected safe dose (determined from previous data from long-term feeding studies conducted in rats and dogs). No doses exceeding 1000 mg/kg-bw per day were used. It was reported that data from the F_2 litter showed no indication of adverse effects on reproductive performances (Pierce et al. 1974). Additional studies: International Research and Development Corp. (1974); Litton Bionetics (1981b). No reproductive toxicity studies were identified for the dermal and inhalation routes of exposure.
Developmental toxicity	*Oral* Rats (unspecified strain, unspecified number of pregnant animals per group) were exposed to Solvent Red 48 via gavage during the organogenesis period of the pups (exact exposure period and dose frequency were not specified). Doses were based on highest no-effect levels in rats and dogs in prior 2-year feeding studies (no further details provided in the abstract). No evidence of skeletal or soft tissue abnormalities in the fetuses were reported (Burnett et al. 1974). No additional oral developmental toxicity studies were identified. No developmental toxicity studies were identified for the dermal and inhalation routes of exposure.
Genotoxicity and related endpoints: *in vitro*	Positive, with and without metabolic activation (S9), in *Salmonella typhimurium* strains TA97, TA98, TA100, TA102, TA104, TA1535 and TA1537 for mutations (NTP 2002). Inconclusive for bacterial DNA repair in *Bacillus subtilis strains* H17 and M45 (GENETOX 1991). No other *in vitro* genotoxicity studies were identified in the literature.
Genotoxicity and related endpoints: *in vivo*	No *in vivo* genotoxicity studies were identified in the literature.
Humans	

[8] Although the original study of Hazleton Laboratories (1969) was not available and only limited information is provided in the secondary reference (NTP 2000), it is believed that these are the same data reported in Carson et al. (1984).

Endpoint	**Lowest effect levels/Results** (see notes 1–5 at end of this table)
Epidemiological studies	No epidemiological studies were identified in the literature.

Notes:
1. LC_{50} = median lethal concentration; LD_{50} = median lethal dose; LOEC = lowest-observed-effect concentration; LOEL = lowest-observed-effect level.
2. Dietary conversion factor for rats (% in diet to mg/kg per day): 1% in food = 10 000 ppm = 500 mg/kg per day (Health Canada 1994).
3. Dietary conversion factor for mice (% in diet to mg/kg per day): 1% in food = 10 000 ppm = 1300 mg/kg per day (Health Canada 1994).
4. Dietary conversion factor for dogs (% in diet to mg/kg per day): 1% in food = 10 000 ppm = 300 mg/kg per day (Health Canada 1994).
5. Dermal conversion factor: 1 mg applied to skin 2x weekly = applied dose of approximately 9.5 mg/kg-bw per day (= 2 mg/week = 0.29 mg/day, body weight of rat = 0.30 kg) (Health Canada 1994).

Appendix 6 – Summary of Health Effects Information for Phloxine B (Sodium Salt)

Endpoint	**Lowest effect levels/Results** (see notes 1 and 2 at end of this table)
Laboratory animals and *in vitro* assays	
Acute toxicity	Lowest iv LD_{50} = 310 mg/kg-bw in mice (Lutty 1978). No oral, inhalation, dermal or LD_{50} values were identified in the literature.
Skin irritation	Rabbits (New Zealand albino white, 1 male, 2 females) were exposed to Phloxine B in 0.1 ml of water topically at 0.5 g to the intact left flank for 4 hrs under semi-occlusion. The scoring of skin reactions was performed at 1, 24, 48 and 72 hours, as well as 7, 10 and 14 days after removal of the dressing. Based upon the classification criteria (Commission Directive 2001/59/EC), Phloxine B was concluded to be not irritating to skin (SCCNFP 2004). Rabbits (New Zealand albino white, 1 male, 2 females) were exposed to Phloxine B in 0.1 ml of water topically at 100 mg into one eye. Scoring of irritation effects was performed at approximately 1, 24, 48 and 72 hours, as well as 7 and 10 days and 14 and 17 days, post-application. Based on the classification criteria (Commission Directive 2001/59/EC), Phloxine B was concluded to be not irritating to the eye (SCCNFP 2004). Mice (CBA/J, 4 female mice per dose) were exposed to Phloxine B topically at concentrations of 0, 0.5, 1.5, 3.0 or 7.0 % (in DMSO). On days 0, 1 and 2 the experimental animals received 25 µl of the Phloxine B solution, positive control or vehicle on the dorsal surface of each pinnae. All animals were sacrificed on day 5 for assessment of cell proliferation. The investigators concluded that the test substance is not a skin sensitizer (SCCNFP 2004). No other irritation or sensitization studies have been identified in the literature. The SCCNFP concluded that Phloxine B is not a skin or eye irritant and will not pose a sensitizing risk to consumers when used as intended.
Short-term repeated-dose toxicity	*Oral* **LOEL** = 250 mg/kg-bw per day (NOEL = 50 mg/kg-bw per day) for exposure-related clinical chemistry effects in rats. Rats (HanBrl:WIST(SPF), 5 animals per sex per group) were exposed to Phloxine B via water (10 ml/kg-bw per day) at levels of 0, 10, 50 or 250 mg/kg-bw per day (doses cited in SCCNFP 2004), 7 days per week for 4 weeks. No exposure-related effects on body weight, food intake, organ weights or mortality were reported. Exposure-related decreases in basophils and increases in platelet counts were reported in the high-dose females. Furthermore, irritation of the stomach was reported in the animals exposed at either 50 (dyskeratosis) or 250 (focal spongiosis of the limiting ridge) mg/kg-bw per day (SCCNFP 2004). No other oral studies were identified in the literature. No short-term dermal or inhalation toxicity studies were identified in the literature.
Subchronic toxicity	*Oral* **LOEL** = 250 mg/kg-bw per day (NOEL = 50 mg/kg-bw per day) for clinical chemistry effects in male rats. Rats (HanBrl:WIST(SPF), 5 animals per sex per group) were exposed to Phloxine B via water (10 ml/kg-bw per day) at levels of 0, 10, 50 or 250 mg/kg-bw per day (doses cited in SCCNFP 2004), 7 days per

Endpoint	**Lowest effect levels/Results** (see notes 1 and 2 at end of this table)
	week for 13 weeks. One male and one female died (at 50 mg/kg-bw per day, as result of dosing error). No exposure-related effects on body weight, food intake, organ weights or ophthalmoscopy were reported. Myosis and decreased locomotor activity were reported in the high-dose females and males, respectively. While an increased absolute eosinophil count in high-dose females was reported, decreased triglycerides, protein levels, and alanine amino transferase (ALAT) activity were reported in equally dosed males. Impaired concentrating ability (increased volume and decreased density) was detected upon urine analysis in high-dose males and females. Furthermore, increased urinary pH in high-dose males and red colouration of the urine was reported in mid- and high-dose groups. Passive discolouration of various segments of the digestive tract in all exposed animals and stomach irritation in both sexes at 250 mg/kg-bw per day (vacuolation limiting ridge epithelium, hyaline inclusions in glandular mucosa and squamous hyperplasia in most males and females, and submucosal cell infiltrate in all females) were reported. Squamous hyperplasia was also observed in males exposed to Phloxine B at 50 mg/kg-bw per day. 10 mg/kg-bw per day was established by the authors as the no-observed-adverse-effect level (NOAEL). However, the SCCNFP suggested that the NOAEL should be at 50 mg/kg-bw per day, assuming that the signs of stomach irritation represent a local effect due to irritating potential of the test substance (in the mid-dose group), and that the mortalities were due to dosing errors (SCCNFP 2004). No subchronic inhalation or dermal toxicity studies were identified in the literature.
Chronic toxicity/ carcinogenicity	*Oral:* **Lowest oral LOEL** = 130 mg/kg-bw per day for increased liver weights. Mice (C57BL/6N x C3H/HeN, 49–64 males, 46–62 females per group) were exposed to Phloxine B via diet at concentrations of 0, 0.1 or 0.4 % (approximately 0, 130 or 520 mg/kg-bw per day; see note 2 at end of Appendix for dose conversion) starting at 6 weeks of age for a maximum of 90 weeks. Survival rates of the exposed animals were at 85%, 64 weeks after the start of Phloxine B exposures. The incidence of tumours such as skin (fibrosarcoma, papilloma or lipoma), lung (carcinoma or adenoma), lymphoma (systemic or localized), splenic hematoma, gastric papilloma, intestinal adenoma, liver (carcinoma, adenoma or altered foci), hemangioma, adrenocortical adenoma, ovary (granulosa cell tumour, hemangioma), mammary gland (carcinoma or hemangioma), harderian gland tumour, testis (germinoma or embryonal carcinoma) and uterus (myosarcoma, adenocarcinoma, hemangioma or lipoma) was not significantly increased in exposed animals when compared to the controls. However, increased incidence of pituitary tumours (1.6% (1/62) in control group, 20.4% (10/49) at low dose, 13.0% (6/46) at high dose) was reported in female mice. No comment on the pituitary tumours was provided, and no data were given concerning variation in historical controls of the study. Statistically increased liver weights above controls were reported for males of both treatment groups and in low-dose females. However, no liver histopathology was reported other than liver tumours, which did not show any treatment-related differences except that altered liver foci were significantly reduced in the high-dose males. In addition, contrary to other studies on Phloxine B, the body weights of males and females of both dose groups were increased above controls throughout the study (from months 4–16, up to > 20% of control weight in female dose groups). Although there was no apparent difference in body weights at termination, the study authors reported a “massive increase” in adipose tissues of exposed mice upon autopsy (data not reported), which was explained by a possible improved feed palatability of Phloxine B. A “diffuse red appearance” of major organs

Endpoint	**Lowest effect levels/Results** (see notes 1 and 2 at end of this table)
	indicates that at least some absorption of (and therefore systemic exposure to) Phloxine B had occurred in this study. The study authors concluded that the data clearly indicated that subchronic or chronic oral administration of Phloxine B did not have any tumorigenic effects in mice of either sex. A review of this data by the SCCNFP (2004) did not render conclusions on the relevance of the pituitary tumours or liver weights, and only indicated the study to be not adequately reported (Ito et al. 1994). No inhalation or dermal carcinogenicity studies were identified in the available literature.
Reproductive and developmental toxicity	*Oral* **Oral LOEL** = 1300 mg/kg-bw per day (lowest tested dose; a NOEL was not established) based on split cervical arches in pups. Pregnant Jcl:ICR mice were given Phloxine B in the diet at concentrations of 0, 1, 3 and 5% (equivalent to 0, 1300, 3900 and 6500 mg/kg-bw per day; see note 2 at end of Appendix for dose conversion) from the morning of day 6 through day 16 of gestation. The mice were killed on day 18 and fetuses were examined for external, visceral and skeletal anomalies. A significant decrease in body-weight gain was observed in all of the treated groups. Among the dams in the high-dose group, two maternal deaths, one abortion and a significant increase in liver weight were observed. A dose-related incidence of splitting of the cervical vertebral arches (nos. 3–6) was noted in all of the treated groups, but this anomaly was not found in the controls. The total incidence of skeletal anomalies was also dose-related and was significantly increased at the 3 and 5% dose levels. Maternal toxicity was observed in all doses as evidenced by decreased maternal weight gain in all dose groups, while the high-dose groups showed increased liver weights (absolute and relative) and increased mortality relative to controls. It was concluded that Phloxine B was teratogenic in mice at dietary levels of 3 and 5%, levels which resulted in maternal toxicity, and that a finding suggesting a teratogenic effect (split cervical arches) occurred at the 1% dose level. The study authors suggested that since individual fetuses with split cervical arches did not correlate with specific dams' weight decrease (data not shown), maternal toxicity was not responsible for this teratogenic effect (Seno et al. 1984). Rats (HanBrl:WIST(SPF), 5 mated females) were exposed to Phloxine B via water at levels of 0, 10, 50 and 250 mg/kg-bw per day (doses cited in SCCNFP 2004) during days 6–20 of gestation. Slight reductions in food consumption and body weight were reported in the high-dose animals. No teratogenic effects were reported (SCCNFP 2004). In a subsequent study, rats (HanBrl:WIST(SPF), 5 mated females) were exposed to Phloxine B via water at levels of 0, 10, 50 and 250 mg/kg-bw per day during days 6–20 of gestation. No exposure-related effect on mortality was reported. Transient reductions (slight) in body-weight gain and food intake were reported in the high-dose animals. No teratogenic effects were reported (SCCNFP 2004). The investigators concluded that Phloxine B elicited slight maternal toxicity at 250 mg/kg-bw per day but was not embryotoxic or teratogenic at any of the doses tested. NOAEL = 50 mg/kg-bw per day for maternal toxicity was determined by the SCCNFP 2004 (SCCNFP 2004). Other oral developmental studies: Nakaura et al. (1975), Uchida and Enomoto (1971). No developmental toxicity studies were identified in the literature for the dermal and inhalation routes of exposure.

Endpoint	**Lowest effect levels/Results** (see notes 1 and 2 at end of this table)
Genotoxicity and related endpoints: *in vitro*	Negative for gene mutations in *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and TA102 in the presence and absence of metabolic activation (SCCNFP 2004). Negative for gene mutation in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 in the presence and absence of metabolic activation (Maus et al. 1981). Negative for mutations in mouse lymphoma L5178 cells of the thymidine kinase locus in the presence and absence of metabolic activation (SCCNFP 2004). Positive for reverse mutations in *Escherichia coli* B/r WP2 (Kada et al. 1972). Negative for reverse mutations in *Escherichia coli* WP2 (Maus et al. 1981). Positive for mutations in *Bacillus subtilis strains rec*$^{-}$ *and rec*$^{+}$ following UV irradiation (Kada et al. 1972). Positive for mutations in *Bacillus subtilis strains rec*$^{-}$ *and rec*$^{+}$ following UV irradiation via fluorescent bulb (Yoshikawa et al. 1978). Positive for DNA damage in *Bacillus subtilis* 168 met^{-} cells in the presence of UV radiation (Yoshikawa et al. 1978). Positive for oxidative damage to cellular RNA and DNA in human skin fibroblasts in the presence of fluorescent light (Wei et al. 1995). No other *in vitro* genotoxicity studies were identified in the literature.
Genotoxicity and related endpoints: *in vivo*	Negative for clastogenicity in the bone marrow of mice (NMRI, 2 animals per sex per group) exposed to Phloxine B via intraperitoneal injection at dose levels of 25, 50, 100, 150 or 200 mg/kg-bw (SCCNFP 2004). No other *in vivo* genotoxicity studies were identified in the literature.
Humans	
Epidemiological studies	No epidemiological studies were identified in the literature.

Notes:
1. LC_{50} = median lethal concentration; LD_{50}, = median lethal dose; LOEC = lowest-observed-effect concentration; LOEL = lowest-observed-effect level.
2. Dietary conversion factor for mice (% in diet to mg/kg per day): 1% in food = 10 000 ppm = 1300 mg/kg per day (Health Canada 1994).