

Draft Screening Assessment

Triarylmethanes Group

Chemical Abstracts Service Registry Numbers

548-62-9

569-64-2

1324-76-1

2390-59-2

2390-60-5

3844-45-9

**Environment and Climate Change Canada
Health Canada**

December 2018

Synopsis

Pursuant to section 68 or 74 of the *Canadian Environmental Protection Act, 1999* (CEPA), the Minister of the Environment and the Minister of Health have conducted a screening assessment on six substances referred to collectively under the Chemicals Management Plan as the Triarylmethanes Group. These six substances were identified as priorities for assessment as they met categorization criteria under subsection 73(1) of CEPA or were considered a priority on the basis of other concerns. A seventh substance was initially included in the group; however, it was determined to be of low concern through other approaches, and the proposed conclusion for this substance is provided in a separate report¹. Accordingly, this screening assessment addresses the six substances listed in the table below. The six substances addressed in this screening assessment will hereinafter be referred to as the Triarylmethanes Group.

Substances in the Triarylmethanes Group

CAS RN ^a	<i>Domestic Substances List</i> name	Common name
548-62-9 ^b	Methanaminium, N-[4-[bis[4-(dimethylamino)phenyl]methylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, chloride	Basic Violet 3
569-64-2	Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, chloride	Malachite Green
1324-76-1 ^b	Benzenesulfonic acid, [[4-[[4-(phenylamino)phenyl][4-(phenylimino)-2,5-cyclohexadien-1-ylidene]methyl]phenyl]amino]-	Pigment Blue 61
2390-59-2	Ethanaminium, N-[4-[bis[4-(diethylamino)phenyl]methylene]-2,5-cyclohexadien-1-ylidene]-N-ethyl-, chloride	Basic Violet 4
2390-60-5	Ethanaminium, N-[4-[[4-(diethylamino)phenyl][4-(ethylamino)-1-naphthalenyl]methylene]-2,5-cyclohexadien-1-ylidene]-N-ethyl-, chloride	Basic Blue 7

¹ Proposed conclusions for CAS RN 632-99-5 are provided in the Rapid Screening of Substances with Limited General Population Exposure Draft Screening Assessment.

CAS RN^a	<i>Domestic Substances List</i> name	Common name
3844-45-9 ^b	Benzenemethanaminium, N-ethyl-N-[4-[[4-[ethyl[(3-sulfophenyl)methyl]amino]phenyl](2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-3-sulfo-, hydroxide, inner salt, disodium salt	Brilliant Blue FCF

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^b This substance was not identified under subsection 73(1) of CEPA but was included in this assessment as it was considered a priority on the basis of other human health concerns.

Triarylmethanes are primarily used as colouring agents and do not occur naturally in the environment. They are used as dyes and/or pigments in inks, toners, and colourants, in paper products and manufactured items, and potentially in food packaging materials, for commercial and consumer use. Substances in this group are also used in other products available to consumers including children's arts and crafts materials (e.g., paint markers), cosmetics (e.g., body cream, hair products, hair dyes, makeup, perfume), cleaning products, and water treatment for aquarium fish, as well as in additional industrial and laboratory products. Specifically, Basic Violet 3 is a medicinal ingredient in drugs for human and veterinary use. Brilliant Blue FCF is also used in food, natural health products, pest control products, prescription and non-prescription drugs and a range of additional products available to consumers. All six substances were imported into Canada in quantities for each substance ranging from 1000 to 100 000 kg while Brilliant Blue FCF was manufactured in Canada in a quantity ranging from 100 to 1000 kg, according to information submitted for the reporting years of either 2008 or 2011.

All substances in the Triarylmethanes Group may be released to the Canadian environment on the basis of their uses in Canada including paper dyeing, deinking of paper, as well as from the formulation, manufacture and consumer use of products containing these substances. Releases are expected to the aquatic environment from both diffuse and point sources. Releases of some of these substances to terrestrial environments are also possible. If released to the aquatic environment, Pigment Blue 61 is likely to behave like a particle and settle to bed sediment. The other triarylmethane substances will be charged at environmentally relevant pH and will tend to sorb to dissolved and suspended solids. Therefore, these substances may potentially be transported in the water column or settle to bed sediment. Substances in the Triarylmethanes Group tend to persist in water, sediment and soil. They have a low potential to bioaccumulate in the lipids of aquatic organisms; however, the non-sulfonated dyes (i.e., Basic Violet 3, Basic Violet 4, Malachite Green and Basic Blue 7) instead may bind to proteins and accumulate in other types of fish tissue.

Experimental acute toxicity data for the non-sulfonated dyes show they have the potential to cause adverse effects to aquatic organisms at low concentrations. Adverse effects in aquatic organisms were observed for Brilliant Blue FCF at relatively higher

concentrations, whereas no effects were observed at the solubility limit for Pigment Blue 61. Ecological exposure scenarios were developed for down-the-drain releases from uses of products containing these substances and for releases from industrial sites. Risk quotient analyses were conducted to compare estimated aquatic concentrations to adverse effect concentrations in aquatic organisms for different exposure scenarios. Scenarios for paper dyeing and paper deinking indicate that the non-sulfonated triarylmethane dyes pose a risk to aquatic organisms, whereas the scenarios for general formulation/product handling and consumer uses did not. Exposure scenarios for Brilliant Blue FCF did not show a risk to aquatic organisms and Pigment Blue 61 is not expected to pose a risk for aquatic organisms as it is expected to behave more like a particle and would not likely be bioavailable.

Considering all available lines of evidence presented in this draft screening assessment, there is risk of harm to the environment from Basic Violet 3, Malachite Green, Basic Violet 4, and Basic Blue 7. It is proposed to conclude that Basic Violet 3, Malachite Green, Basic Violet 4, and Basic Blue 7 meet the criteria under paragraph 64(a) of CEPA as they are entering or may enter 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. However, it is proposed to conclude that Basic Violet 3, Malachite Green, Basic Violet 4, and Basic Blue 7 do not meet the criteria under paragraph 64(b) of CEPA as they are not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger to the environment on which life depends. It is also proposed to conclude that Pigment Blue 61 and Brilliant Blue FCF do not meet the criteria under paragraphs 64(a) or (b) of CEPA as they are 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.

For the general population of Canada, the predominant sources of exposure to dye substances in the Triarylmethanes Group are from use of products available to consumers that contain these substances, and from environmental media (e.g., drinking water). Potential oral exposures to Basic Violet 3, Malachite Green, Basic Violet 4, Basic Blue 7 and Brilliant Blue FCF were estimated based on potential levels in drinking water. Potential dermal and oral exposures to Brilliant Blue FCF were derived from use of natural health products and cosmetics, as well as oral exposures from its use as a food additive. Potential dermal and/or oral exposures to Malachite Green, Basic Violet 4 and Basic Blue 7 were derived from use of paint markers (a children's arts and crafts product) and/or cosmetics (hair dyes). Potential inhalation exposure to Brilliant Blue FCF from use of perfume was also characterized. Inhalation exposure to the remaining substances in the Triarylmethanes Group was not considered to be of concern due to their negligible volatility, as well as their potential uses. Exposure to Pigment Blue 61 for the general population of Canada is not expected on the basis of its physical and chemical properties and identified uses.

In laboratory studies, Basic Violet 3 is not observed to cause developmental or reproductive toxicity, but is genotoxic and can increase liver tumours. On the basis of health effects information for a structurally-related substance, the critical health effect for Malachite Green is developmental toxicity. Pigment Blue 61 was not identified as posing a high hazard to human health on the basis of classifications by other national or international agencies for carcinogenicity, genotoxicity, developmental toxicity or reproductive toxicity. Basic Violet 4, and the structurally-related substance Basic Blue 7, are not genotoxic. On the basis of health effects information on structurally-related substances, Basic Violet 4 and Basic Blue 7 are not considered to be developmental or reproductive toxicants, but may be carcinogenic. Brilliant Blue FCF is poorly absorbed orally and dermally, is not a developmental or reproductive toxicant, is not genotoxic, and is not carcinogenic.

For Basic Violet 3, Basic Violet 4, Basic Blue 7, and Brilliant Blue FCF, comparisons of levels of exposure to the general population and levels at which critical health effects were observed results in margins of exposure considered adequate to address uncertainties in the health effects and exposure databases for both non-cancer and cancer effects. In contrast, similar comparisons of exposure from use of Malachite Green in hair dye and paint markers resulted in inadequate margins of exposure, particularly since the critical health effects were observed at the lowest tested dose.

On the basis of the information presented in this draft screening assessment, it is proposed to conclude that Malachite Green meets the criteria under paragraph 64(c) of CEPA as it is entering or may enter 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 also proposed to conclude that Basic Violet 3, Pigment Blue 61, Basic Violet 4, Basic Blue 7, and Brilliant Blue FCF do not meet the criteria under paragraph 64(c) of CEPA as they are 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.

Therefore, it is proposed to conclude that Basic Violet 3, Malachite Green, Basic Violet 4, and Basic Blue 7 meet one or more of the criteria set out in section 64 of CEPA. It is proposed to conclude that Pigment Blue 61 and Brilliant Blue FCF do not meet any of the criteria set out in section 64 of CEPA. It is also proposed that Basic Violet 3, Malachite Green, Basic Violet 4, and Basic Blue 7 meet the persistence criteria but not the bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations* of CEPA.

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1. Introduction

Pursuant to section 68 or 74 of the *Canadian Environmental Protection Act, 1999* (CEPA) (Canada 1999), the Minister of the Environment and the Minister of Health have conducted a screening assessment on six of seven substances, referred to collectively under the Chemicals Management Plan as the Triarylmethanes Group, to determine whether these six substances present or may present a risk to the environment or to human health. These six substances were identified as priorities for assessment as they met categorization criteria under subsection 73(1) of CEPA or were considered a priority on the basis of other concerns (ECCC, HC [modified 2017]).

The seventh substance, CAS RN² 632-99-5, was originally included in the Triarylmethanes Group. However, it was considered in the Ecological Risk Classification of Organic Substances (ERC) Science Approach Document (ECCC 2016), and via the approach applied in the Rapid Screening of Substances with Limited General Population Exposure (ECCC, HC 2017), and it was identified as being of low concern to both human health and the environment. As such, it is not further addressed in this report. Proposed conclusions for this substance are provided in the Rapid Screening of Substances with Limited General Population Exposure Draft Screening Assessment (ECCC, HC 2017). The six substances addressed in this draft screening assessment will hereinafter be referred to as the Triarylmethanes Group.

While all the substances in the Triarylmethanes Group have common structural features and similar functional uses as pigments or dyes in multiple sectors, there is notable diversity within the group with respect to overall structure and physical chemical properties. This diversity has been taken into account through the individual assessment of each substance.

Certain substances within the Triarylmethanes Group currently being evaluated were reviewed internationally through the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the European Food Safety Authority (EFSA), the US Environmental Protection Agency (US EPA), the European Chemicals Agency (ECHA), the National Toxicology Program (NTP), and there are existing assessments available. These assessments undergo rigorous review. Health Canada and Environment and Climate Change Canada consider these assessments to be reliable.

This draft screening assessment includes consideration of information on chemical properties, environmental fate, hazards, uses and exposures, including additional information submitted by stakeholders. Relevant data were identified up to April 2017. Empirical data from key studies as well as some results from models were used to

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reach proposed conclusions. When available and relevant, information presented in assessments from other jurisdictions was considered.

This draft screening assessment was prepared by staff in the CEPA Risk Assessment Program at Health Canada and Environment and Climate Change Canada and incorporates input from other programs within these departments. The ecological and human health portions of this assessment have undergone external review and/or consultation. Comments on the technical portions relevant to the environment were received from Dr. Isabel Beauchesne, Mr. Geoff Granville (GCGranville Consulting Corp.), and Dr. Jarai Mon. Comments on the technical portions relevant to human health were received from Ms. Theresa Lopez, Ms. Jennifer Flippin, and Dr. Joan Garey at Tetra Tech. While external comments were taken into consideration, the final content and outcome of the screening assessment remain the responsibility of Health Canada and Environment and Climate Change Canada.

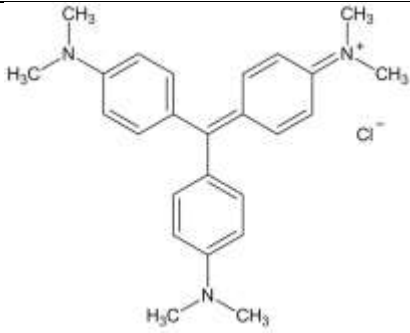
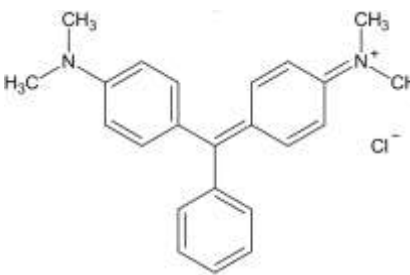
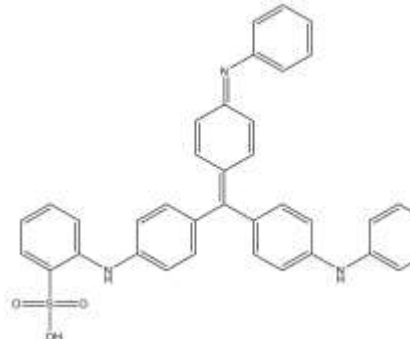
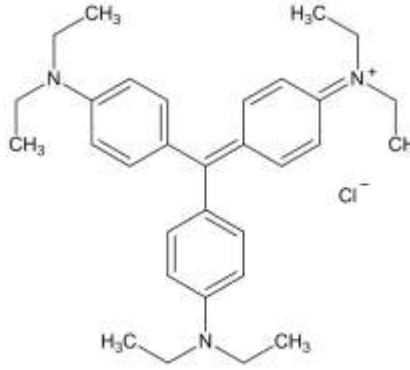
This draft screening assessment focuses on information critical to determining whether substances meet the criteria as set out in section 64 of CEPA, by examining scientific information and incorporating a weight-of-evidence approach and precaution.³ This draft screening assessment presents the critical information and considerations on which the proposed conclusions are based.

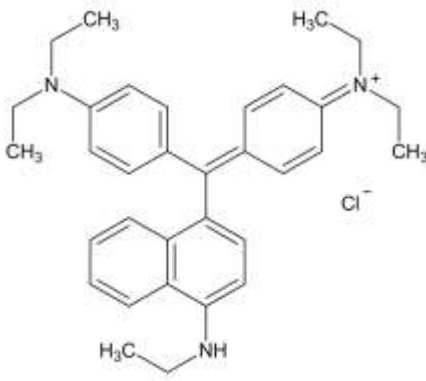
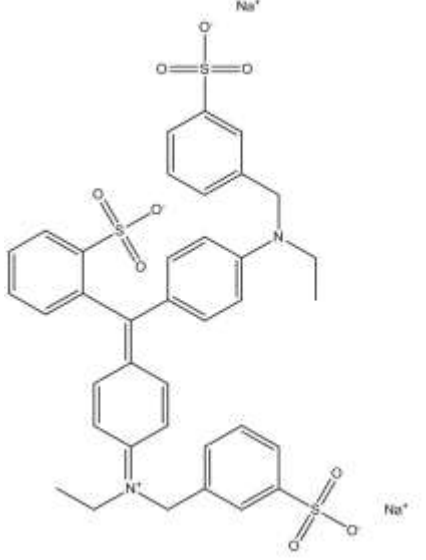
2. Identity of substances

The Chemical Abstracts Service Registry Numbers (CAS RN), *Domestic Substances List* (DSL) names and common names for the substances in the Triarylmethanes Group are presented in Table 2-1.

³A determination of whether one or more of the criteria of section 64 of CEPA 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 products available to consumers. A conclusion under CEPA is not relevant to, nor does it preclude, an assessment against the hazard criteria specified in the *Hazardous Products Regulations*, which are part of the regulatory framework for the Workplace Hazardous Materials Information System for products intended for workplace use. Similarly, a conclusion based on the criteria contained in section 64 of CEPA does not preclude actions being taken under other sections of CEPA or other acts.

Table 2-1. Substance identities^a

CAS RN	DSL name (common name)	Chemical structure and molecular formula	Molecular weight (g/mol)
548-62-9	Methanaminium, N-[4-[bis[4-(dimethylamino)phenyl]methylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, chloride (Basic Violet 3 ^b)	 $C_{25}H_{30}N_3 Cl$	407.99
569-64-2	Methanaminium, N-[4-[[4-(dimethylamino)phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-N-methyl-, chloride (Malachite Green)	 $C_{23}H_{25}N_2 Cl$	364.92
1324-76-1	Benzenesulfonic acid, [[4-[[4-(phenylamino)phenyl][4-(phenylimino)-2,5-cyclohexadien-1-ylidene]methyl]phenyl]amino]- (Pigment Blue 61)	 $C_{37}H_{29}N_3 O_3 S$	595.72
2390-59-2	Ethanaminium, N-[4-[bis[4-(diethylamino)phenyl]methylene]-2,5-cyclohexadien-1-ylidene]-N-ethyl-, chloride (Basic Violet 4)	 $C_{31}H_{42}N_3 Cl$	492.15

CAS RN	DSL name (common name)	Chemical structure and molecular formula	Molecular weight (g/mol)
2390-60-5	Ethanaminium, N-[4-[[4-(diethylamino)phenyl][4-(ethylamino)-1-naphthalenyl]methylene]-2,5-cyclohexadien-1-ylidene]-N-ethyl-, chloride (Basic Blue 7)	 $C_{33}H_{40}N_3Cl$	514.15
3844-45-9	Benzenemethanaminium, N-ethyl-N-[4-[[4-[ethyl[(3-sulfophenyl)methyl]amino]phenyl](2-sulfophenyl)methylene]-2,5-cyclohexadien-1-ylidene]-3-sulfo-, hydroxide, inner salt, disodium salt (Brilliant Blue FCF)	 $C_{37}H_{34}N_2O_9S_3Na_2$	792.86

^a Colour index (C.I.) numbers (Sigma-Aldrich c2017): Basic Violet 3: 42555; Malachite Green: 42000; Basic Violet 4: 42600; Basic Blue 7: 42595; Pigment Blue 61: 42765:1; Brilliant Blue FCF: 42090.

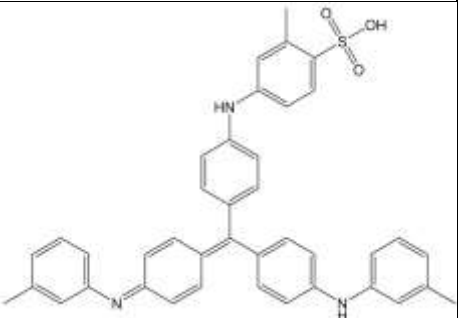
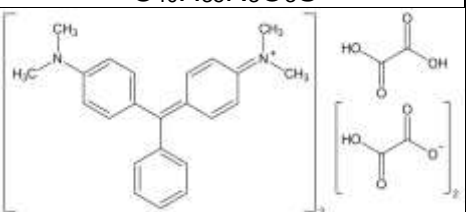
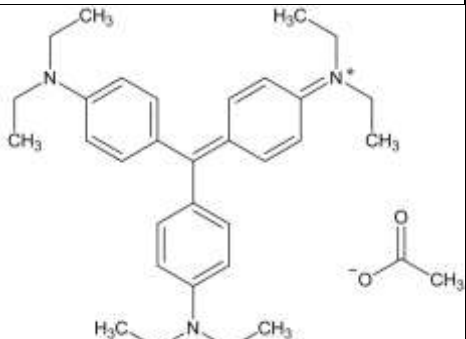
^b Basic Violet 3 (CAS RN 548-62-9) is commonly referred to in the literature as crystal violet, and gentian violet. The term "gentian violet" originally was used to describe a mixture of methyl pararosaniline dyes (methyl violet), but is now commonly used to refer to the single component Basic Violet 3. Toxicology studies in this document using the term "gentian violet" were commercial preparations of at least 96% CAS RN 548-62-9, with the remainder being mainly methyl violet or pentamethylpararosaniline (Aidoo et al. 1990).

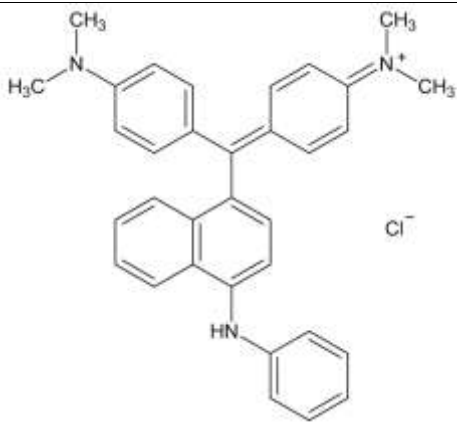
2.1 Selection of analogues and use of (Q)SAR models

A read-across approach using data from analogues and the results of (quantitative) structure-activity relationship ((Q)SAR) models, where appropriate, has been used to

inform the ecological and human health assessments. Analogues were selected that were structurally similar and/or functionally similar to substances within this group (similar physical-chemical properties, toxicokinetics), and that had relevant empirical data that could be used to read-across to substances with limited empirical data. The applicability of (Q)SAR models was determined on a case-by-case basis. Details of the read-across data and (Q)SAR models chosen are further discussed in the Ecological Effects Assessment and Health Effects Assessment sections of this report. Information on the identities of the analogues used to inform this assessment is presented in Table 2-2.

Table 2-2. Analogue identities^a

CAS RN	Common name	Chemical structure and molecular formula	Molecular weight (g/mol)	Target substance(s) for analogue
6417-46-5	Pigment Blue 56	 $C_{40}H_{35}N_3O_3S$	637.80	Pigment Blue 61
2437-29-8	Malachite Green Oxalate	 $C_{46}H_{50}N_4 \cdot C_2H_2O_4 \cdot 2C_2HO_4$	927.02	Malachite Green
63157-72-2	Ethyl Violet Acetate	 $C_{31}H_{42}N_3 \cdot C_2H_3O_2$	515.74	Basic Violet 4, Basic Blue 7

CAS RN	Common name	Chemical structure and molecular formula	Molecular weight (g/mol)	Target substance(s) for analogue
2580-56-5	Basic Blue 26	 $C_{33}H_{32}N_3 \cdot Cl$	506.09	Basic Blue 7

^a Additional substances within the Triarylmethanes Group (e.g., Basic Violet 3, Malachite Green) were also used as analogues. Their use is identified in the applicable section.

3. Physical and chemical properties

A summary of physical and chemical property data of the substances in the Triarylmethanes Group is presented in Table 3-1 and Table 3-2.

When experimental information was limited or not available, (Q)SAR models were used to generate data for vapour pressure, Henry's law constant, octanol-water partition coefficient (K_{ow}), organic carbon-water partition coefficient (K_{oc}) and octanol-air partition coefficient (K_{oa}). Many of these models are mainly based on fragment addition methods (i.e., they rely on the structure of the chemical) and typically accept only the neutral (i.e., un-ionized) form of a chemical as input (in SMILES form). As such, the un-ionized form was used as model input where required. Where more than one appropriate model or valid empirical result was available for a given property, the mean was taken as the key value for that parameter. The selected key values for the estimation of vapour pressure, water solubility, $\log K_{ow}$, air-water partition coefficient ($\log K_{aw}$), and $\log K_{oa}$ were adjusted using the least-squares adjustment procedure (Cole and Mackay 2000; Schenker et al. 2005), and represent internally consistent partitioning properties considering thermodynamic constraints.

The four dyes Basic Violet 3, Malachite Green, Basic Violet 4 and Basic Blue 7 are chloride salts. As salts they will dissociate in water to produce the corresponding cationic organic dye moiety and chloride counterion. These substances are oxidized to charged species during their synthesis, and are expected to retain this positive charge in the environment. The pKa values have not been reported as they are not applicable

to these permanent charges, which would be present over the whole pH range. In addition to the permanent charge, the speciation of these substances in biological fluids will also be dependent on pH (ACD/Percepta c1997-2015).

Brilliant Blue FCF is a disodium salt with three sulfonate groups in its structure. At pH 4 and above, greater than 95% of the molecules will have a single amine group that will be positively charged and all three sulfonic acid groups will be negatively charged, resulting in a net negative charge (ACD/Percepta c1997-2015).

In contrast to the other triarylmethanes, Pigment Blue 61 is expected to behave more like a particle similar to other organic pigments. The substance has a median particle diameter size of 294 μm (ECHA c2007-2017a). The physical and chemical properties of many of the structural classes of pigments are often not amenable to model prediction because they are typically considered out of the model domain of applicability (e.g., structural and/or property parameter domains). Due to the molecular structure features, organic pigments tend to have very low solubility. Therefore the octanol-water partition coefficient for sparingly soluble substances is reasonably represented by the quotient of solubilities in octanol and in water ($S_{\text{oct}}/S_{\text{w}}$) (ECHA 2008). For Pigment Blue 61, the log ($S_{\text{oct}}/S_{\text{w}}$) value was estimated to be 3.99 based on a water solubility of 2.5 $\mu\text{g/L}$ and octanol solubility of 23.8 mg/L .

Table 3-1. Summary of physical and chemical property values (at standard temperature) for Basic Violet 3, Malachite Green, Basic Violet 4, and Basic Blue 7

Property	Basic Violet 3	Malachite Green	Basic Violet 4	Basic Blue 7	Reference(s)
Adjusted water solubility (mg/L)	1.8×10^4	1.3×10^4	6.7×10^3	2.1×10^4	ACD/Percepta c1997-2015; Baughman et al. 1994; Green 1990; SCBT c2007-2016; WATERNT 2010; WSKOWWIN 2010
Experimental water solubility (mg/L)	4.0×10^3	4.0×10^4	9.0×10^3	2.0×10^4	Baughman et al. 1994; Green 1990; SCBT c2007-2016
Vapour pressure (Pa) ^a	1.8×10^{-10}	3.6×10^{-9}	3.3×10^{-11}	4.0×10^{-13}	MPBPWIN 2008
Henry's law constant (Pa·m ³ /mol)	2.0×10^{-12}	1.2×10^{-10}	1.1×10^{-11}	3.9×10^{-14}	HENRYWIN 2008
Log K_{aw} (dimensionless)	-14.8	-13.4	-15.0	-17.4	Schenker et al. 2005
Adjusted log K_{ow} (dimensionless)	0.7	0.6	4.7	4.8	ACD/Percepta, c1997-2015; KOWWIN 2010

Property	Basic Violet 3	Malachite Green	Basic Violet 4	Basic Blue 7	Reference(s)
Experimental log K _{ow} (dimensionless)	0.5	0.6	2.4	NA	ECHA c2007-2017b; Hansch et al. 1995; Tsai et al. 1991
Log K _{oc} (dimensionless)	1.2	1.2	3.4	3.5	KOCWIN 2010
Log K _{oa} (dimensionless)	15.5	14.0	19.7	22.3	KOAWIN 2010

Abbreviation: NA: Not available

^a As triarylmethane substances are solids at room temperature, subcooled liquid vapour pressures are calculated.

Table 3-2. Summary of physical and chemical property values (at standard temperature) for Pigment Blue 61 and Brilliant Blue FCF

Property	Pigment Blue 61	Brilliant Blue FCF	Reference(s)
Vapour pressure (Pa) ^a	N/A	3.0×10 ⁻⁴²	MPBPWIN 2008
Henry's law constant (Pa·m ³ /mol)	N/A	1.4×10 ⁻³¹	HENRYWIN 2008
Adjusted water solubility (mg/L)	N/A	1.8×10 ³	ECHA c2007-2017a; Green 1990; ACD/Percepta c1997-2015; WATERNT 2010; WSKOWWIN 2010
Experimental water solubility (mg/L)	2.5×10 ⁻³	3.0×10 ⁴	ECHA c2007-2017a; Green 1990
Solubility in octanol (mg/L)	23.8	NA	ECHA c2007-2017a
Log K _{aw} (dimensionless)	N/A	-38.4	Schenker et al. 2005
Adjusted log K _{ow} (dimensionless)	N/A	0.3	ACD/Percepta c1997-2015; KOWWIN 2010
Log K _{ow} (dimensionless)	3.99 ^b	0.02	ACD/Percepta c1997-2015; ECHA c2007-2017a; KOWWIN 2010
Log K _{oc} (dimensionless)	< 1.3–4.9	0.3	ECHA c2007-2017a; KOCWIN 2010
Log K _{oa} (dimensionless)	N/A	37.3	KOAWIN 2010

Abbreviations: N/A: Not applicable, NA: Not available

^a As triarylmethane substances are solids at room temperature, the subcooled liquid vapour pressures are calculated.

^b The log K_{ow} for pigments is estimated by calculating the ratio between the empirical solubility in octanol and the empirical solubility in water.

4. Sources and uses

None of the substances in the Triarylmethanes Group occur naturally. These substances have been included in surveys issued pursuant to section 71 of CEPA for the reporting years 2008 (Basic Violet 3, Malachite Green) or 2011 (Pigment Blue 61, Basic Violet 4, Basic Blue 7, Brilliant Blue FCF) (Canada 2009, 2012). Brilliant Blue FCF was reported to be manufactured in Canada in a quantity ranging from 100 to 1000 kg in the 2011 calendar year (Environment Canada 2013). All six substances were imported into Canada in quantities for each substance ranging from 1000 to 100 000 kg, according to section 71 surveys for reporting years of either 2008 or 2011 (Environment Canada 2009, 2013).

In Canada and globally, the six substances of the Triarylmethanes Group are primarily used as colourants, spanning a wide variety of potential applications. Table 4-1 and Table 4-2 present a summary of the uses of these substances in Canada.

Table 4-1. Summary of Canadian uses of substances in the Triarylmethanes Group (on the basis of information obtained from CEPA section 71 surveys)

Major uses ^a	BV3	MG	PB61	BV4	BB7	BBFCF
Agricultural substances (non-pesticidal)	N	N	N	N	N	Y
Arts, crafts and hobby materials (including children's uses)	N	N	N	N	N	Y
Cleaning and furnishing care	N	N	N	N	N	Y
Food and beverage	N	N	N	N	N	Y
Ink, toner and colourants	N	Y	Y	N	N	Y
Laundry and dishwashing	N	N	N	N	N	Y
Lawn and garden care	N	N	N	N	N	Y
Medical devices	Y ^b	N	N	N	N	N
Paint and coatings	N	N	N ^c	N	N ^c	Y
Paper products, mixtures or manufactured items	Y	Y	N	Y	N	N
Personal care products	N	N	N	N	N	Y

Abbreviations: BV3 = Basic Violet 3, MG = Malachite Green, PB61 = Pigment Blue 61, BV4 = Basic Violet 4, BB7 = Basic Blue 7, BBFCF = Brilliant Blue FCF, Y = yes this use was reported for this substance, N = no this use was not reported for this substance

^a Non-confidential uses reported in response to the surveys conducted under section 71 of CEPA (Environment Canada 2009, 2013). See surveys for specific inclusions and exclusions (schedules 2 and 3).

^b Reported as a laboratory substance only.

^c Although not reported in section 71 surveys, this substance is known to be used in Canada in paints and coatings, but at volumes below the CEPA section 71 survey reporting threshold of 100 kg/year (personal communication, emails from the Canadian Paints and Coatings Association to Environment and Climate Change Canada, 2016-2017; unreferenced).

Table 4-2. Additional uses in Canada identified for each of the substances in the Triarylmethanes Group

Use	BV3	MG	PB61	BV4	BB7	BBFCF
Arts, crafts and hobby materials (including children's uses)	N	Y ^a	N	N	N	N
Food additive ^b	N	N	N	N	N	Y ^c
Food packaging materials ^{b,d}	Y	Y	Y	Y	Y	Y
Incidental additives ^b	N	N	N	N	N	Y ^e
Formulant in pest control products registered in Canada ^f	N	N	N	N	N	Y ^g
Internal Drug Product Database as medicinal or non-medicinal ingredients in disinfectant, human or veterinary drug products in Canada ^h	Y ⁱ	N	N	N	N	Y ^j
Natural Health Products Ingredients Database ^k	Y ^l	N	N	N	N	Y ^m
Licensed Natural Health Products Database as medicinal or non-medicinal ingredients in natural health products in Canada ^k	N	N	N	N	N	Y ⁿ
Notified to be present in cosmetics, on the basis of notifications submitted under the <i>Cosmetic Regulations</i> to Health Canada ^o	N	Y ^p	N	Y ^q	Y ^r	Y ^s
Inks (including printing ink) ^t	Y	Y	Y	Y	Y	Y
Water treatment for aquarium fish	N	Y ^u	N	N	N	N

Abbreviations: BV3 = Basic Violet 3, MG = Malachite Green, PB61 = Pigment Blue 61, BV4 = Basic Violet 4, BB7 = Basic Blue 7, BBFCF = Brilliant Blue FCF, Y = yes this use was indicated for this substance, N = no this use was not indicated for this substance

^a May be available in Canada from use in paint markers based on publicly available information; MSDS 2014.

^b Personal communication, emails from the Food Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, 2016-2017; unreferenced.

^c Health Canada [modified 2015a].

^d Potentially used in food packaging materials.

^e Identified as a possible component in incidental additives (e.g., cleaners and dish detergents) used in food processing plants.

^f Personal communication, email from the Pest Management Regulatory Agency, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, 2017; unreferenced.

^g PMRA 2010.

^h Personal communication, email from the Therapeutic Products Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, 2016; unreferenced.

ⁱ Identified as a Medicinal Ingredient (i.e., Active Ingredient (AI)); DPD [modified 2015].

^j Identified as a Non-Medicinal Ingredient (NMI) in a wide variety of prescription and non-prescription drugs including allergy medications and cold medications; Canada 1978.

^k Personal communication, emails from the Natural and Non-prescription Health Products Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, 2016 - 2017; unreferenced.

^l Listed in the Natural Health Products Ingredients Database (NHPID) with a non-NHP role because not a naturally occurring substance included in Schedule 1 of the *Natural Health Products Regulations* (NHPID [modified 2017]).

^m Listed in the NHPID with a non-medicinal role for use as a colour additive in natural health products (NHPID [modified 2017]). Also associated with an acceptable daily intake of up to 6 mg/kg bw/day based on JECFA (2017).

ⁿ Listed in the Licensed Natural Health Products Database as being present as non-medicinal ingredient in a variety of currently licensed natural health products, including workout supplements, multi-vitamin/mineral supplements, acne therapy products, and toothpastes (LNHPD [modified 2016]).

- ° Personal communication, emails from the Consumer Product Safety Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, 2016-2017; unreferenced.
- p Identified in semi-permanent hair dye.
- q Identified in semi-permanent hair dyes.
- r Identified in semi-permanent hair dye and hair conditioner.
- s Identified in a wide variety of cosmetics including body cream, various hair products, makeup, and perfume.
- t General use assumed to be in Canada; Herbst and Hunger 2004; Hunger 2003.
- u May be available in Canada from use in water treatment for aquarium fish based on publicly available information; Hikari USA 2016.

Basic Violet 3 and Malachite Green are listed on the Pest Management Regulatory Agency (PMRA) List of Active Pesticide Ingredients, and Malachite Green and Basic Blue 7 (as well as Brilliant Blue FCF, as indicated above in Table 4-2) are listed on the PMRA Pesticide Formulants List, but neither Basic Violet 3, Malachite Green, nor Basic Blue 7 are present in any currently registered pest control products in Canada (Personal communication, email from the Pest Management Regulatory Agency, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, 2017; unreferenced; PMRA 2010).

Internationally, Basic Violet 3 has previously been reported as a colourant in cosmetics (Diamante et al. 2009; AGDH 2014; EWG c2007-2017). However, in Canada it is an ingredient that is currently listed as prohibited on the Cosmetic Ingredient Hotlist (Health Canada [modified 2015b])⁴ and has also been prohibited from use in cosmetics, including hair dyes, in Europe (EC 2009). In Europe, Malachite Green is also prohibited from use in cosmetics, and Basic Violet 4 and Basic Blue 7 are prohibited from use in hair dye products in Europe (EC 2009). Triarylmethane dyes and pigments are generally recognized for their use internationally in the printing inks industry, particularly for use in packaging (Herbst and Hunger 2004), for the dyeing of paper and textiles, and for their use in cosmetics, drugs, and food (Hunger 2003). Basic Violet 3 and Malachite Green also have laboratory uses as pH indicators and biological stains (Hunger 2003).

5. Releases to the environment

Malachite Green was reported to the National Pollutant Release Inventory by a single company involved in chemical manufacturing with ≤ 0.004 tonnes per year released to all environmental media between 2003 and 2007 (NPRI 1993-2015).

There are potential releases of substances in the Triarylmethanes Group to water from industrial facilities involved in deinking of paper and paper dyeing (for substances

⁴ The List of Prohibited and Restricted Cosmetic Ingredients (more commonly referred to as the Cosmetic Ingredient Hotlist or simply the Hotlist) is an administrative tool that Health Canada uses to communicate to manufacturers and others that certain substances, when present in a cosmetic, may contravene the general prohibition found in section 16 of the *Food and Drugs Act* (FDA) or a provision of the *Cosmetic Regulations*. Section 16 of the FDA states that "No person shall sell any cosmetic that has in or on it any substance that may cause injury to the health of the user". In addition, the Hotlist includes certain substances that may make it unlikely for a product to be classified as a cosmetic under the FDA (Health Canada [modified 2015b]).

associated with these uses), as well as the formulation or manufacture of products and consumer use of products containing these substances. Down-the-drain releases to wastewater treatment systems⁵ (WWTSs) and eventually to surface water could result from various uses of products available to consumers that contain these substances.

Intentional applications on soil of fertilizers or pesticides containing Brilliant Blue FCF could result in releases to surface water through run-off.

6. Environmental fate and behaviour

6.1 Environmental distribution

The substances in the Triarylmethanes Group are not expected to be released to air given their intended uses and physical-chemical properties. These substances have very low vapour pressure and Henry's law constant, and they exist in a cationic form in the environment (with the exception of Brilliant Blue FCF and Pigment Blue 61). These properties indicate that volatilization would be negligible from soil surfaces and surface waters. As such, long-range atmospheric transport is not expected to occur.

Given the reported uses of substances in the Triarylmethanes Group, it is expected that these substances may end up in surface water. The characteristics of the WWTS and the affinity of the triarylmethane substances for dissolved and suspended solids will determine the degree to which these substances end up in surface water. Generally, most of the substances in the Triarylmethanes Group are expected to be associated with dissolved and suspended solids to a large degree. Although the characteristics of Brilliant Blue FCF make it less likely to sorb to particles in the environment, some sorption may occur depending on the conditions of the media (German-Heins and Flury 2000).

Pigment Blue 61 has very low solubility, while the other substances are all soluble in water. Within the aquatic environment, Pigment Blue 61 is likely to behave like a particle and settle to bed sediment whereas the other substances will dissociate, releasing the ionic triarylmethane molecule and the associated counterion. The non-sulfonated dyes will exist as cations at environmentally relevant pH, and thus will have an affinity for negatively charged particles in the water column (e.g., humic and fulvic acids, clay materials) although a fraction of them may remain in the water column. The sorption processes would be dominated by electrostatic interactions as a result of the negatively

⁵ In this assessment, the term "wastewater treatment system" (WWTS) refers to a system that collects domestic, commercial and/or institutional household sewage and possibly industrial wastewater (following discharge to the sewer), typically for treatment and eventual discharge to the environment. Unless otherwise stated, the term WWTS makes no distinction of ownership or operator type (municipal, provincial, federal, indigenous, private, partnerships). Systems located at industrial operations and specifically designed to treat industrial effluents are identified by the terms "on-site WWTSs" and/or "industrial WWTSs."

charged sorption sites on dissolved organic carbon and suspended solids, although organic carbon may also play a small role (Kah and Brown 2006; Droge and Goss 2012, 2013). Brilliant Blue FCF will also exist in a charged state (having a net negative charge); therefore, its environmental fate will also be dictated by electrostatic interactions. Transport of these dyes in water may occur and suspended solids may eventually settle to bed sediment, where the sorbed dyes are likely to remain unless mixing and transport of the bed sediment occurs. Therefore, the non-sulfonated triarylmethane dyes will likely bind to particulate matter and settle to sediment, whereas, due to the high solubility and the anionic character of Brilliant Blue FCF, it is more likely to remain in the water.

The fate of the triarylmethane substances in soils will also be determined by their sorption characteristics. Due to the high solubility and the anionic character of Brilliant Blue FCF, under certain conditions, this dye could move through the soil pore water. Pigment Blue 61 is not expected to be mobile, given its poor water solubility. The non-sulfonated triarylmethane dyes are not expected to be very mobile either. While ion-exchange is complex and not fully understood (Droge and Goss 2012, 2013), it is expected that triarylmethane dyes would have an affinity for charged particles and may or may not be mobile depending on the moisture content, soil type, and amount of soil erosion or runoff. For example, the non-sulfonated triarylmethane dyes would likely be less mobile in soils with high organic matter or high clay content (Droge and Goss 2012, 2013; Kah and Brown 2006). In addition, for organic cations such as the non-sulfonated triarylmethane dyes, the sorption affinity further depends on competition with other organic cations present in soils (Droge and Goss 2012). Also, Brilliant Blue FCF adsorption appears to be influenced by the ionic strength of soil solution (German-Heins and Flury 2000).

6.2 Environmental persistence

ETAD (1995) states that, with some exceptions, dyes may be considered essentially non-biodegradable under aerobic conditions. Repeated evaluation of ready- and inherent-biodegradability of over 80 different dyes using accepted screening tests (e.g., OECD tests) have confirmed this characteristic (Pagga and Brown 1986; ETAD 1992). Although there is some evidence that triarylmethane dyes will degrade over time (Bumpus and Brock 1988; Andrews et al. 1990; Perez-Estrada et al. 2008; Ogugbue and Sawidis 2011), modelling information (BIOWIN 2008) seems to show that the biodegradation of triarylmethanes will be relatively slow with extrapolated half-lives (where applicable) ranging from weeks to months and some being considered to be recalcitrant.

Similar to other organic pigments, Pigment Blue 61 is not expected to biodegrade in aquatic systems (ECHA c2007-2017a).

Therefore, the substances in the Triarylmethanes Group are expected to be persistent in environmental media (water, sediment and soil), with predicted half-lives greater than 182 days for water and soil, and greater than 365 days for sediment (BIOWIN 2008).

Thus, the potential for organisms to be exposed both spatially and temporally to these substances in the environment is increased.

6.3 Potential for bioaccumulation

The empirical information on Malachite Green and the modelled information for the other substances in the group indicate that these triarylmethanes are not likely to bioaccumulate in aquatic organisms (Table 6-1). However, due to the cationic nature of the non-sulfonated triarylmethane dyes, the K_{ow} and octanol solubility, which are used to estimate the BCF/BAF values from models such as EPIWIN BCFBAF (2010), may not be appropriate predictors of bioaccumulation for these substances. For these triarylmethane dyes, partitioning to proteins in the cell membrane is more likely to occur than to lipids, the latter being estimated using K_{ow} and octanol solubility values.

Table 6-1. Empirical and estimated data for bioconcentration of substances in the Triarylmethanes Group

Common name	Test organism	Experimental concentration mg/L (duration)	BCF (L/kg)	BAF (L/kg)	Reference
Basic Violet 3	Fish	NA	3.2	1.4	BCFBAF 2010
Malachite Green	Carp	0.002–0.02 (56 days)	75–91	NA	NITE 2002
Basic Violet 4 ^a	Fish	NA	1091	1191	BCFBAF 2010
Basic Blue 7 ^a	Fish	NA	2104	2717	BCFBAF 2010
Pigment Blue 61	Fish	NA	476 ^b	NA	ECHA c2007-2017a
Brilliant Blue FCF	Fish	NA	3.2	0.9	BCFBAF 2010

Abbreviations: BCF, bioconcentration factor; BAF, bioaccumulation factor; NA, Not Available

^a Estimated mid-trophic BCF/BAF including biotransformation rate estimates (Arnot-Gobas method) as estimated log K_{ow} values are greater than 4.

^b The BCF is calculated as the concentration in biota (1.19 mg/L) divided by the concentration in water (0.0025 mg/L), following the method of Gobas and Morrison (2000).

There is indication that the potential accumulation of triarylmethanes in the flesh of fish would likely be through binding to protein and DNA (Docampo and Moreno 1990; Mani and Bharagava 2016). This is supported by the fact that some of the substances in the Triarylmethanes Group are commonly used as biological stains in laboratories because they easily stain amino acids within proteins (Mani and Bharagava 2016).

When solubilised in natural water systems, the non-sulfonated triarylmethane dyes could bind to the surface of various tissues (e.g., fish gills, algae, dermal surfaces) or to food items. Although it is unclear if these triarylmethane dyes would cross the gastrointestinal tract to the bloodstream, it is reasonable to estimate that a fraction of them could bind to plasma protein (Enoch et al. 2011). Malachite Green has been

shown to persist in fish tissue (Xie et al. 2012; Srivasta et al. 2004; Lanzing 1965; Poe and Wilson 1983).

Although triarylmethane substances (specifically, Malachite Green) have been measured in the flesh of fish (Jiang et al. 2008; Zhijun et al. 2010), they are likely to be depurated from the body due to their physical-chemical properties (Bergwerff et al. 2004; Niska et al. 2009).

Brilliant Blue FCF is likely to behave differently than the non-sulfonated triarylmethane dyes as it is expected to be negatively charged (anion) at environmentally relevant pH and thus is less likely to partition to cell membranes as cationic substances can. Moreover, being highly soluble in water, it is not expected to partition to storage lipids in fish, therefore it is not expected to bioaccumulate in aquatic organisms.

In contrast to the triarylmethane dyes in the group, Pigment Blue 61 is a neutral substance at environmentally relevant pH; therefore, it is not expected to interact electrostatically with various media. As such, an estimate of the log K_{ow} can reliably be calculated and thus be used to estimate a BCF value. An average fish lipid content of 5% (Geyer et al. 1985) is commonly used to normalize whole-body lipid content in recognized guidelines (OECD TG 305). The solubility of Pigment Blue 61 in octanol of 23.8 mg/L (Table 3-2) and the average fish lipid content of 5% was used to estimate a maximum concentration of the substance in fish of 1.19 mg/L. Thus, the BCF value for Pigment Blue 61, calculated following the method of Gobas and Morrison (2000), is 476 (Table 6-1). As such, Pigment Blue 61 is not expected to bioaccumulate in fish (Anliker and Moser 1987).

Overall, information on Brilliant Blue FCF and Pigment Blue 61 indicate that these substances have low potential for bioaccumulation. Available experimental data and modelled results indicate that the non-sulfonated triarylmethane dyes may have some potential for bioaccumulation, though at levels well below the criteria as set out in the *Persistence and Bioaccumulation Regulations* of CEPA. The non-sulfonated triarylmethane dyes are not expected to bioaccumulate significantly in the lipid tissues of aquatic organisms; however, they can interact and bind with proteins which may result in some bioaccumulation in other tissues.

7. Potential to cause ecological harm

7.1 Ecological effects assessment

7.1.1 Mode/mechanism of action

Using the OECD Toolbox, the profiler description for the mode of action of these triarylmethane substances is reactive unspecified. In addition, some outcomes of the ToxCast AR binding model indicate the potential for endocrine-type effects. Substances in the Triarylmethanes Group seem to cause three main types of cellular effects.

First, there is evidence that they cause mitochondrial disturbance. Basic Violet 3 appears to concentrate in animal mitochondria where it disrupts these organelles by acting as an uncoupler of oxidative phosphorylation (Docampo and Moreno 1990). It can increase ATPase activity, release respiratory control and interfere with ATP synthesis (Docampo and Moreno 1990). Similarly, studies have found that Malachite Green can damage mitochondria and cause nuclear alteration (Gerundo et al. 1991) which can result in increased glycolysis, a situation which is common in cancer cells (Xu et al. 2005).

Second, triarylmethanes may act through binding to DNA and proteins. Studies have reported that Basic Violet 3 binds with DNA and interacts with two adjacent A-T base pairs, causing kinking, severe bending or unwinding of the DNA double helix. The result is chromosomal alteration and damage (Docampo and Moreno 1990). Triarylmethanes are commonly used as biological stains in laboratories since they easily stain amino acids within proteins (Mani and Bharagava 2016).

Third, triarylmethanes may cause adverse effects through free radical damage in cells. Basic Violet 3 can be photo-reduced into a carbon-centered reactive oxygen species (ROS) through exposure to visible light (Docampo et al. 1988). Under aerobic conditions, a photodynamic action occurs, during which the free radical auto-oxidizes. It is believed that this photo-reduction action is mediated by oxygen reduction products (Docampo et al. 1988). Malachite Green has also been shown to trigger depletion of intracellular iron pools in organisms and, similarly to Basic Violet 3, enhance ROS levels (Dhamgaye et al. 2012). There is evidence to suggest that excessive accumulation of ROS can lead to necrosis (Xu et al. 2005).

Triarylmethanes may also cause adverse effects in organisms through physical effects. Malachite Green has been shown to increase ventilation and respiration rates in fish, as it clogs the gills with particulates, increases production of mucus and causes epithelial damage (Ross et al. 1985).

In general, due to the similarity in physical-chemical characteristics, the four non-sulfonated triarylmethane dyes are expected to have a common mechanism of action when pertaining to ecological harm

While there is a lack of data on the effects of Basic Violet 4 and Basic Blue 7, they are expected to act similarly to Basic Violet 3 and Malachite Green, given their similarity in structure. Brilliant Blue FCF and Pigment Blue 61, however, are expected to have lower toxicity due to the presence of sulfonic acid (SO₃) groups. Studies have observed that increasing the number of SO₃ groups on a molecule make the molecules more hydrophilic and will decrease the substance's toxicity (Mon et al. 2006).

7.1.2 Effects on aquatic organisms

While the acute toxicity of Basic Violet 3 and Malachite Green to aquatic organisms has been well characterized, limited data are available for Brilliant Blue FCF and there is a

lack of aquatic toxicity data for Basic Violet 4, Basic Blue 7, and Pigment Blue 61. In light of the similarities in the non-sulfonated triarylmethane dyes, the toxicity information available on Basic Violet 3 and Malachite Green were used as read-across to assess the toxicity of Basic Violet 4 and Basic Blue 7.

Ecological effects studies available for these substances include data for fish, invertebrates, and algae. Based on the available data, which are primarily from acute studies, the most sensitive organisms appear to be freshwater fish. The key studies for aquatic organisms can be found in Appendix A (Table A-1).

The critical toxicity value selected for aquatic organisms for the four non-sulfonated dyes (i.e., Basic Violet 3, Malachite Green, Basic Violet 4 and Basic Blue 7) was a 96-hour LC₅₀ of 0.03 mg/L from a study that examined the toxicity of Malachite Green on Bluegill fish (Bills et al. 1977). An assessment factor of 30 was applied to account for the acute to chronic extrapolation (factor of 10) and to address uncertainty around substances that are expected to have a reactive mode of action (factor of 3). No extrapolation to account for interspecies variation was required because there are effects data available for a large number of species (i.e., greater than 10) from several different taxonomic groups. The PNEC for non-sulfonated dyes was therefore calculated as 0.001 mg/L. This value indicates that Basic Violet 3, Malachite Green, Basic Violet 4 and Basic Blue 7 (both individually and collectively) have the potential to cause adverse effects to aquatic organisms at low concentrations.

Although no reproduction or developmental data were found for aquatic organisms, there is evidence that Malachite Green Oxalate is a developmental toxicant in mammals (see section 8.2).

For Brilliant Blue FCF, one empirical aquatic toxicity study was available. The most sensitive endpoint in the study was a 96-hour LC₅₀ of 180 mg/L for sockeye salmon (Wan et al. 1991). As the dye tested in the study was 50% Brilliant Blue FCF, it is estimated that the LC₅₀ for Brilliant Blue FCF would be half this value, i.e., an LC₅₀ of 90 mg/L, assuming toxicity is expected to increase linearly and that the other components (surfactant and other formulants) did not contribute to the overall toxicity of the tested substance. An assessment factor of 600 was applied to extrapolate from short-term lethal median effects to long-term sub-lethal low effects (factor of 10), to account for interspecies variation because the dataset consisted of 6 species from one taxonomic group (factor of 20), and to account for the reactive mode of action for this substance (factor of 3). The resulting PNEC value derived for this substance was 0.15 mg/L.

The aquatic studies for both Pigment Blue 61 and its analogue, Pigment Blue 56, demonstrated no effects at concentrations that are well above their solubility limits (2.5 µg/L), indicating that at its most dissolved state, Pigment Blue 61 would not be expected to cause any adverse effects. Therefore, a PNEC for Pigment Blue 61 was not derived.

7.1.3 Effects on sediment and soil organisms

Data for soil and sediment toxicity of substances in the Triarylmethanes Group are very limited. A soil toxicity study available for Malachite Green reported a 14-day LC₅₀ value of 1.45 mg/kg for earthworms (Gopinathan et al. 2015). Triarylmethane dyes that are used as biological stains (such as Malachite Green and Basic Violet 3) can act as a DNA binder and protein binder. This action could potentially cause adverse effects leading to death in skin breathing organisms like earthworms by hindering the respiratory functions of the skin. This was shown to be the case for earthworms exposed to a xanthene dye, which is a substance that also binds to DNA and protein (Princz et al. 2014).

Given the limited ecotoxicity data and exposure characterization of these substances (discussed in the next section), PNECs for soil and sediment were not derived for any of the substances in the Triarylmethanes Group.

7.2 Ecological exposure assessment

Potential environmental exposure of organisms to substances in the Triarylmethanes Group will occur mainly through surface water. No environmental monitoring data were available for any of these substances; therefore exposures were estimated for key scenarios. Exposure characterization was focused on the scenarios that represent the most probable exposure situations for the four non-sulfonated dyes collectively and for Brilliant Blue FCF. These scenarios included paper dyeing, paper deinking, general formulation, and consumer uses resulting in down-the-drain releases to WWTSSs. For all four scenarios, a probabilistic approach was used.

Table 7-1. Summary of exposure scenarios considered

Scenario	Description of scenario	Substances included
1	Paper dyeing	4 non-sulfonated substances (Basic Violet 3, Malachite Green, Basic Violet 4, and Basic Blue 7)
1	Paper dyeing	Brilliant Blue FCF
2	Paper de-inking	4 non-sulfonated substances, as above
2	Paper de-inking	Brilliant Blue FCF
3	General formulation	4 non-sulfonated substances, as above
4	Consumer uses	Brilliant Blue FCF

Releases of the non-sulfonated dyes to surface water are expected to occur via industrial WWTSSs for scenarios 1 and 2, and both on-site and off-site WWTSSs for scenario 3. Given their physical-chemical properties, they will partition to sludge to a large degree. As sludge from on-site WWTSSs would not be applied to agricultural or pasture lands, an exposure scenario for soil was not developed for the non-sulfonated dyes. Brilliant Blue FCF is found in products available to consumers and releases to surface water are expected to occur via WWTSSs. However, given its physical-chemical

properties (lower affinity for suspended solids) and the lack of soil toxicity data to derive a soil PNEC, an exposure scenario for soil was not developed for this substance. Exposure scenarios were not developed for Pigment Blue 61 because it has very low water solubility, effects on aquatic organisms were only observed well above its water solubility limit in the presence of an emulsifier, and there were no data available for soil-dwelling organisms. It is expected to behave as a particle given its median particle size and low water solubility, hence it likely would not be bioavailable.

7.2.1 Calculation of PECs and general assumptions

Predicted environmental concentrations (PECs) were calculated collectively for the four non-sulfonated dyes combined (Basic Violet 3, Malachite Green, Basic Violet 4, Basic Blue 7) and for Brilliant Blue FCF for relevant exposure scenarios identified. The industrial release scenarios were based on the maximum production capacities of the facilities, rather than use quantities reported for individual substances. It was assumed that any one of the non-sulfonated triarylmethane dyes could be substituted for another. Therefore, no distinction was made between the different substances, and a single collective PEC range for non-sulfonated triarylmethane dyes was generated. The PECs represent potential concentrations of these substances in the receiving water body near the discharge point of a WWTS. The PEC values are presented in each exposure scenario and a summary of key assumptions are provided in Appendix B.

In all cases, aquatic PECs were derived using a range of removal rates applicable to the type of treatment technologies employed at the WWTS and based on the physical-chemical properties of the triarylmethane substances. All aquatic PECs were also derived using a dilution factor based on the 10th percentile flow rate of the receiving water body and capped at 10.

7.2.2 Scenario 1: paper dyeing

In this scenario, 39 pulp and paper mills in Canada that have the capability to dye paper were considered. Two PECs were developed, one for paper dyeing using any of the four non-sulfonated triarylmethane dyes, and the other for paper dyeing using Brilliant Blue FCF.

Information was compiled for each of these facilities, including site data for known production capacities, operating days, water discharge rates, receiving water body flow rates, and on-site wastewater treatment technologies employed. Other key parameters that are not specific to a particular site were estimated as distributional ranges and applied to all sites. These parameters included dye usage rate, fraction of the substance in the dye mixture, the retention rate of the dye, and removal rate for a given treatment type. Refer to Appendix B (Table B-1) for a summary of assumptions. These parameters were used in a Monte Carlo analysis resulting in a range of PECs. These predictions were then compared to the acute whole-effluent testing results available for these sites. Results for one site were eliminated because the modelled PECs were

above LC_{50s} for aquatic organisms, and yet acute toxicity tests with the whole effluent showed no adverse effects.

For any of the non-sulfonated triarylmethane dyes, the calculated PECs in receiving water bodies near the point of discharge range from 3.6×10^{-6} to 515 µg/L, with 35% of iterations yielding PECs greater than the PNEC (1 µg/L).

For Brilliant Blue FCF, the calculated PECs in receiving water bodies near the point of discharge range from 3.2×10^{-5} to 626 µg/L. In this case, less than 1% of iterations yield PECs greater than the PNEC (150 µg/L).

7.2.3 Scenario 2: paper deinking

This scenario considered the removal of inks containing triarylmethane dyes from recycled paper. Two PECs were calculated, one for the four non-sulfonated dyes and one for Brilliant Blue FCF with the assumption that some of the recycled paper was printed with ink containing one or more of the four non-sulfonated dyes or Brilliant Blue FCF.

Recycled paper may contain triarylmethane dyes both in the paper fiber from dyes used to colour the paper itself, as well as in the inks printed on the paper. It was assumed that the deinking process removes just the ink (and the dye it contains) affixed to the surface of the paper and not the dye bound to the paper fiber that was used to colour the paper (Liu et al. 2007). A key assumption is the mass of the substance in the paper to be recycled which was estimated using the capacity of a given deinking plant. Additional assumptions about the composition of recycled paper included the average coverage of ink (15 to 25%; tonercartridgedepot.com c1998-2017), the average paper density (45 to 105 g/m²; OKI 2017), and the ink millage or coverage (0.2 to 0.5 m²; AOPI 2017). The resulting fractional ink content of a given pile of paper bound for recycling was estimated between 0.004 and 0.01 gram of ink per gram of paper (g ink/g paper). This estimate is supported by European estimates of ink content in paper for recycling which range from 0.003 to 0.07 g ink/g paper (OECD 2009).

Thirteen pulp and paper recycling plants were used in the scenario, along with their individual known recycling capacities, effluent flow rates, on-site wastewater treatment systems, and dilution factors. Assumptions included a fractional emission factor of ink (0.02 to 0.2; Rodger Beatson & Associates 2012) and fraction of triarylmethane substance in the ink (1×10^{-4} and 2×10^{-2}) on a weight basis. A key uncertainty in the PEC calculations is the actual proportion of triarylmethane dyes in the ink. A summary of key assumptions are provided in Appendix B (Table B-2). These parameters were used in a Monte Carlo analysis resulting in a range of PECs.

For any of the non-sulfonated triarylmethane dyes, the calculated PECs in receiving water bodies near the point of discharge range from 5.5×10^{-5} to 231 µg/L, with 50% of iterations yielding PECs greater than the PNEC (1 µg/L).

For Brilliant Blue FCF, the calculated PECs in receiving water bodies near the point of discharge range from 1.8×10^{-6} to 136 $\mu\text{g/L}$. In this case, none of the iterations yield PECs greater than the PNEC (150 $\mu\text{g/L}$).

7.2.4 Scenario 3: general formulation

A probabilistic analysis was conducted to determine PECs for release of triarylmethane dyes from facilities that use these substances in the formulation of products. Given the greater hazard of the four non-sulfonated dyes, this exposure scenario focused on estimating a PEC for general formulation of products containing any of these four substances. Site-specific details of the formulator that reported the largest use quantities of the non-sulfonated triarylmethane dyes in Canada were used. This scenario included details such as mass balance based emission factors ranging from 0.5% to 1.1%, and allowance was given for on-site and off-site secondary removal and high dilution in the environment. If any of these conditions were not present at a facility processing large quantities of non-sulfonated triarylmethane dyes, then refinement of this scenario could impact the outcome and there could be a concern. A summary of assumptions is provided in Appendix B (Table B-3). These parameters were used in a Monte Carlo analysis resulting in a range of PECs.

For any of the non-sulfonated triarylmethane dyes, the calculated PECs in receiving water bodies near the point of discharge range from 3.6×10^{-3} to 2.4 $\mu\text{g/L}$. Less than 2% of iterations yield PECs greater than the PNEC (1 $\mu\text{g/L}$).

7.2.5 Scenario 4: consumer uses

This scenario was developed for Brilliant Blue FCF, given its use in products available to consumers. Consumer release of Brilliant Blue FCF during its use in products is expected to occur throughout Canada. As such, the PEC is estimated using the Consumer Release Aquatic Model (CRAM; Environment and Climate Change Canada Internal Model). CRAM is a Canadian, population-based probabilistic model used to estimate environmental exposure resulting from wastewater treatment facility releases of chemicals present in products available to consumers, which are released down the drain. Distribution information including dilution factors (derived from the 10th percentile flow rate of receiving water bodies), WWTS treatment type and per capita water discharge were used. A wide range of potential chemical usage was used to account for importation of finished products containing these substances.

The calculated PECs for Brilliant Blue FCF in receiving water bodies near the point of discharge range from 3.0×10^{-9} to 455 $\mu\text{g/L}$. Less than 0.01% of PECs are greater than the PNEC (150 $\mu\text{g/L}$).

For a worst case scenario, quantities beyond what was reported under the CEPA section 71 survey were used to account for maximum uses of Brilliant Blue FCF. Relatively high simulated aquatic PECs (above 150 $\mu\text{g/L}$) were largely associated with conditions of consumer usage reaching 3 grams of Brilliant Blue FCF per day per

person, or the total mass of Brilliant Blue FCF reaching 45 million kg per year, which greatly exceeds the total reported mass per year in the Section 71 survey. As an example, given known concentrations of Brilliant Blue FCF in shampoo, even if 100% of Canadians used shampoo containing this substance, the 95th percentile PEC would not exceed the PNEC of 150 µg/L. Although there are other products used by consumers that would also contribute to environmental releases, it is not expected that these would result in significant environmental concentrations, given the low concentrations of Brilliant Blue FCF in these products.

7.3 Characterization of ecological risk

The approach taken in this ecological screening assessment was to examine assessment information and develop proposed conclusions based on a weight-of-evidence approach and using precaution. Evidence was gathered to determine the potential for substances in the Triarylmethanes Group to cause harm in the Canadian environment. Various direct lines of evidence were considered to support the characterization of ecological risk.

7.3.1 Risk quotient analysis

Risk quotient analyses were performed by comparing estimates of exposure (PECs; see the Ecological Exposure Assessment section) with ecological toxicity information (PNECs; see the Ecological Effects Assessment section) to determine whether there is potential for ecological harm in Canada. Risk quotients (RQs) were calculated by dividing the PEC by the PNEC for relevant environmental compartments and associated exposure scenarios. RQs were not calculated for Pigment Blue 61. Table 7-2 presents RQs for the other five triarylmethane dyes for releases to water via wastewater.

Table 7-2. Risk quotient (RQ) calculations for exposure scenarios from wastewater releases of triarylmethane dyes

Exposure scenario	PEC range (µg/L)	Aquatic PNEC (µg/L)	RQ range	Percentage of locations with RQ greater than 1
Paper dyeing (non-sulfonated triarylmethane dyes)	3.6×10^{-6} to 515 ^a	1 ^a	3.6×10^{-6} to 515	35
Paper dyeing (Brilliant Blue FCF)	3.2×10^{-5} to 626	150 ^b	2.1×10^{-7} to 4.2	1

Exposure scenario	PEC range (µg/L)	Aquatic PNEC (µg/L)	RQ range	Percentage of locations with RQ greater than 1
Paper deinking (non-sulfonated triarylmethane dyes)	5.5×10^{-5} to 231 ^a	1 ^a	5.5×10^{-5} to 231	50
Paper deinking (Brilliant Blue FCF)	1.8×10^{-6} to 136	150 ^b	1.2×10^{-8} to 0.91	0
General formulation (non-sulfonated triarylmethane dyes)	3.6×10^{-3} to 2.4 ^a	1 ^a	3.6×10^{-3} to 2.4	2
Consumer uses (Brilliant Blue FCF)	3.0×10^{-9} to 455	150 ^b	2.0×10^{-11} to 3.0	0.01

^a Any of the non-sulfonated triarylmethane dyes (Basic Violet 3, Malachite Green, Basic Violet 4, and Basic Blue 7)

^b Brilliant Blue FCF

With respect to the paper dyeing scenario for the non-sulfonated dyes, simulated RQs above one became far less likely (occurring in only 24% of simulations) when rates of dye use were below 0.04 kg dye per tonne of paper. RQs above one would be much more likely (occurring in 72% of simulations) if rates of dye use were above 0.2 kg dye per tonne of paper. However, often rates of dye use in paper are lower than this at about 0.1 kg dye per tonne of paper (Personal communication, email from the Forest Products Association of Canada, to Environment and Climate change Canada, 2017), which is the rate used in this exposure scenario. At this rate, there could be potential for concern (50% of simulations result in RQs greater than one), but it is uncertain.

Simulated RQs for the paper deinking scenario began showing exceedances of the PNEC when Brilliant Blue FCF was assumed to account for more than 60% of the dyes used in inks on printed paper bound for recycling, a situation that is unlikely to occur. The simulations with non-sulfonated dyes began exceeding the PNEC when these dyes reached 1% of the dyes used in inks on printed paper bound for recycling.

The scenario developed for general formulation and product handling based on the formulator that reported the largest use quantities did not show exceedances of the PNEC due to refinement of site-specific factors such as the mass balance based emission factors, on-site and off-site secondary removal, and high dilution in the environment. However, general formulation and product handling simulations for a generic scenario, considering chemical formulation facilities in Canada that could fill this market demand, showed some potential for exceedances of the PNEC for the non-sulfonated triarylmethane dyes.

7.3.2 Consideration of the lines of evidence

To characterize the ecological risk of substances in the Triarylmethanes Group, technical information for various lines of evidence was considered (as discussed in the relevant sections of this report) and qualitatively weighted. The key lines of evidence supporting the assessment conclusion for the four non-sulfonated triarylmethane dyes are presented in Table 7-3. The level of confidence refers to the combined influence of data quality and variability, data gaps, causality, plausibility and any extrapolation required within the line of evidence. The relevance refers to the impact the line of evidence has when determining the potential to cause harm in the Canadian environment. Qualifiers used in the analysis ranged from low to high, with the assigned weight having five possible outcomes.

Table 7-3. Weighted lines of key evidence considered to determine the potential for Basic Violet 3, Basic Violet 4, Basic Blue 7, and Malachite Green to cause harm in the Canadian environment

Line of evidence	Level of confidence ^a	Relevance in assessment ^b	Weight assigned ^c
Similarity in chemical structure for read-across	High	High	High
Environmental fate and behaviour (ionic nature)	Moderate	High	Moderate to High
Persistence in the environment (i.e., water, sediment, soil)	Moderate	Moderate	Moderate
Bioaccumulation in aquatic organisms	Low	Moderate	Low to Moderate
Mode of action (reactive)	Moderate	Moderate	Moderate
PNEC for aquatic organisms	High	High	High
PECs: paper dyeing	Moderate	High	Moderate to High
PECs: paper deinking	Low	Low	Low
PECs: general formulation and product handling	Moderate	High	Moderate to High
RQs: paper dyeing	Moderate	High	Moderate to High
RQs: paper deinking	Low	Low	Low
RQs: general formulation and product handling	Moderate	High	Moderate to High

^a Level of confidence is determined according to data quality, data variability, data gaps and if the data are fit for purpose.

^b Relevance refers to the impact of the evidence in the assessment.

^c Weight is assigned to each line of evidence according to the combined level of confidence and relevance in the assessment.

A moderate to high level of confidence would be assigned to the data evaluated for Brilliant Blue FCF and Pigment Blue 61, but with low relevance to demonstrating a potential to cause harm in the Canadian environment. These key lines of evidence included their environmental fate and behaviour, persistence, potential for

bioaccumulation, potential to cause adverse effects in aquatic organisms, and RQs that were calculated.

7.3.3 Weight-of-evidence for determining potential to cause harm to the Canadian environment

The four non-sulfonated triarylmethane dyes (Basic Violet 3, Malachite Green, Basic Violet 4, and Basic Blue 7) have similar chemical structures and molecular weights. They are water soluble and have negligible vapour pressure. As such, they are assumed to behave similarly in the environment with respect to both fate in the environment and effects on organisms. If released to the aquatic environment, a fraction of the amount released of these substances may reside in the water column given their water solubility. These substances at environmentally relevant pH are likely to partition to negatively charged, dissolved and suspended solids. This adsorption may or may not be irreversible. Therefore, these substances may be transported in water far from sources or they may settle to bed sediment. Considering the uses of these non-sulfonated triarylmethane dyes, a high degree of removal during on-site wastewater treatment is expected due to partitioning of these dyes to organic matter. As sludge from these on-site WWTSs would not be applied to agricultural or pasture lands, the non-sulfonated triarylmethane dyes would not end up in soil and exposure via this medium would not be a concern.

These four dyes are expected to be persistent in environmental media (water, sediment and soil). Thus, the potential for organisms to be exposed both spatially and temporally to these dyes in the environment is increased. These substances are expected to have a low potential to bioaccumulate in lipid tissues of aquatic organisms based on limited experimental data and modelled results. However, Basic Violet 3 and Malachite Green can interact and bind with proteins and DNA, as evidenced by their functional uses as laboratory stains. Using the OECD Toolbox, the profiler description for the mode of action of triarylmethane substances is reactive unspecified. In addition, outcomes of the ToxCast AR binding model, indicate the potential for endocrine-type effects. However, no empirical information demonstrating such effects was available. The acute toxicity of Basic Violet 3 and Malachite Green to a variety of aquatic organisms is well documented and these substances have been shown to have the potential to cause adverse effects to aquatic organisms at low concentrations ($\mu\text{g/L}$). The most sensitive organisms appear to be freshwater fish.

According to information reported in response to section 71 surveys under CEPA, these triarylmethane substances are mainly used in paper dyeing, inks and toners, and are formulated for use in a wide range of products for consumer and commercial use. Although there is no information on environmental concentrations in the Canadian environment, PECs were calculated for relevant exposure scenarios on the basis of their uses. In comparing PECs with the PNEC for the four non-sulfonated dyes (Basic Violet 3, Basic Violet 4, Basic Blue 7, and Malachite Green), the results for paper dyeing and paper deinking scenarios indicate that these substances pose a risk to aquatic organisms.

Pigment Blue 61 is expected to behave more like a particle, similar to other organic pigments. Considering its physical and chemical properties, it is expected to be persistent in the environment and have a low potential to bioaccumulate in aquatic organisms. The aquatic toxicity studies available for both Pigment Blue 61 and its analogue, Pigment Blue 56, demonstrated no effects at concentrations up to the water solubility limit, with one study showing effects only at concentrations well above the solubility limit with the use of an emulsifier. Therefore an aquatic PNEC was not developed. This substance is not expected to pose a risk for aquatic organisms. No data existed for soil-dwelling organisms; however, considering the properties of pigments, Pigment Blue 61 is expected to be sorbed to soil particles and would not likely be bioavailable.

Brilliant Blue FCF is very soluble in water and is expected to be persistent in the environment. As an anion, it is less likely to bind to sediment or organic matter, has a low potential to bioaccumulate in aquatic organisms and its toxicity to aquatic organisms may be mitigated by its sulfonic acid groups (see, e.g., Table 7.1). However, the PNEC developed is below 1 mg/L due to the relatively high assessment factor used as a result of the limited dataset. The PECs derived for the exposure scenarios show that there is a potential for risk depending on the assumptions used; however, the conditions that would result in RQs above 1 are not likely to be reached. In considering this information, Brilliant Blue FCF is not expected to pose a risk to aquatic organisms.

In summary, the information evaluated for the four non-sulfonated dyes (Basic Violet 3, Malachite Green, Basic Violet 4, and Basic Blue 7) demonstrates that they have the potential to cause ecological harm in Canada, and the information available for Pigment Blue 61 and Brilliant Blue FCF show they have low potential to cause ecological harm in Canada.

It is proposed that Basic Violet 3, Malachite Green, Basic Violet 4, and Basic Blue 7 meet the persistence criteria but not the bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations* of CEPA.

7.3.4 Sensitivity of conclusion to key uncertainties

There were no aquatic toxicity data available for Basic Violet 4 or Basic Blue 7, and the data available for Basic Violet 3 indicated that adverse effects may occur at slightly higher concentrations than those for Malachite Green. The chemical similarities of these four substances warrants using a read-across approach for toxicity to aquatic organisms, and the potential for Malachite Green to cause adverse effects in aquatic organisms is well documented. Therefore, additional empirical toxicity studies for the other substances would not likely change the proposed conclusion. Although there is limited bioaccumulation data for these four dyes, modelled results along with physical-chemical property data corroborate the expected low potential for these substances to bioaccumulate in aquatic organisms. Additional information on bioaccumulative potential would also have a low impact on the proposed conclusion.

There is uncertainty regarding the potential for these substances to cause chronic, reproduction or developmental effects in aquatic species, and the concentrations at which those effects would occur. Although there is evidence that certain triarylmethane dyes bind to proteins and DNA, it is unknown if this could lead to reproduction and/or developmental effects, and empirical information on these types of effects was not available for aquatic organisms. Discussion on the developmental toxicity of Malachite Green to mammals can be found in section 8.2 of this assessment. Depending on the mechanism by which developmental effects occur in mammals, it might be reasonable to expect similar effects in other types of organisms. This uncertainty is addressed through the use of additional assessment factors in deriving PNECs to account for the reactive mode of action of these triarylmethane dyes. Availability of empirical developmental toxicity studies on aquatic organisms could result in refinement of the PNECs.

The exposure scenarios identified for substances in the Triarylmethanes Group are developed on the basis of information obtained through CEPA section 71 surveys, follow-up with stakeholders, and data from the literature. In the absence of particular data, realistic assumptions are made in order to estimate PECs. For the paper dyeing and deinking scenarios, refinement of the dye mass used at sites, the usage rates of the substance(s), emission factors, and fraction of the product containing the actual dye(s) would help increase the confidence in the PECs. In particular for the deinking scenario, better knowledge of how paper recycling plants filter their feedstock (to help estimate the mass of substance) and more reliable information about the emission factors for water-based dyes use in inks could lead to adjustments in this scenario. Sufficient refinement of these factors could impact these scenarios as they may lead to an understanding that exposure is significantly reduced. However, current information is sufficient to support the conclusion that there is a potential to cause ecological harm as a result of the use of the non-sulfonated triarylmethane dyes in paper dyeing and inks.

8. Potential to cause harm to human health

8.1 Exposure assessment

Potential exposures to substances in the Triarylmethanes Group from environmental media, food, and products available to consumers are presented in this section. For each substance, exposure scenarios resulting in the highest exposures were selected to characterize risk.

Environmental media

Substances in the Triarylmethanes Group were not identified or measured in any environmental media in Canada.

The uses of the substances in the Triarylmethanes Group identified through DSL IU survey data (Environment Canada 2009, 2013), information on products submitted to Health Canada, and publicly available data indicate that releases of these substances to

the Canadian environment may result from the consumer use of products containing these substances (i.e., down-the-drain releases) and from various industrial processes. As described in the Ecological Exposure Assessment (see section 7.2), such releases are expected to occur primarily to WWTSs, but treatment technologies may only partially degrade these substances and a portion will partition to biosolids; as such, environmental releases of these substances could contribute to general population exposure through drinking water. Brilliant Blue FCF would be expected to potentially contribute to general population exposure through drinking water as a result of consumer down-the-drain releases whereas the use of the other substances in Canada would not be expected to result in such releases because of the limited or absence of use of these substances in products that would be expected to be poured or washed down-the-drain by consumers. In addition, use of all five dye substances in Canada would be expected to potentially contribute to exposure through drinking water as a result of industrial releases (e.g., from paper dyeing, paper deinking, and general formulation, as noted in section 7.2).

Given the absence of surface monitoring or drinking water data for substances in the Triarylmethanes Group in Canada or elsewhere, theoretical concentrations of each triarylmethane dye in surface water, used as a surrogate for drinking water, were derived from the predicted environmental concentration (PEC) distributions calculated in section 7.2. PEC distributions for potential industrial and consumer release scenarios for the non-sulfonated triarylmethane dyes and Brilliant Blue FCF were considered, where relevant.

The ranges of PECs described in section 7.2 for each of the four release scenarios represent the potential concentrations of the triarylmethane dye substances in a receiving body of water near the discharge point of a WWTS. These scenarios, which were developed for the purpose of the ecological exposure assessment, are anticipated to be overly-conservative within the context of assessing drinking water exposures which would be expected to occur downstream rather than at the point of discharge. One of the key input parameters into the scenarios in section 7.2 was the application of a dilution factor cap of 10; application of such a cap, however, would likely not be representative of a realistic scenario for assessing drinking water exposures further downstream and may result in an overestimate of the concentration of each substance that would be potentially present in drinking water. As such, the 50th percentile PECs for the paper dyeing, paper deinking, and general formulation scenarios were selected (i.e., rather than the upper bound of the ranges described in section 7.2). These scenarios are considered to be more realistic for assessing drinking water exposures while still being conservative.

The 50th percentile PEC for the estimated surface water concentration of Brilliant Blue FCF from consumer releases was used.

The theoretical intake estimates for drinking water for formula-fed infants (0 to 0.5 years) were calculated. The resulting surface water concentrations and theoretical intake estimates for the triarylmethane dyes in drinking water are provided in Table 8-1.

Theoretical concentrations were not estimated for the triarylmethane pigment, Pigment Blue 61, as it is not expected to partition into water.

Table 8-1. Estimated theoretical exposures to the five dye substances in the Triarylmethanes Group from drinking water, based on estimated surface water concentrations

Exposure scenario	Surface water concentration as described in section 7.2 (mg/L)	Exposure from environmental releases, formula-fed infants (mg/kg bw/day) ^b
Paper dyeing (non-sulfonated triarylmethane dyes) ^a	4.9×10^{-4} (50 th percentile)	5.2×10^{-5}
Paper dyeing (Brilliant Blue FCF)	2.8×10^{-3} (50 th percentile)	3.0×10^{-4}
Paper deinking (non-sulfonated triarylmethane dyes) ^a	9.5×10^{-4} (50 th percentile)	1.0×10^{-4}
Paper deinking (Brilliant Blue FCF)	3.7×10^{-4} (50 th percentile)	3.2×10^{-5}
General formulation (non-sulfonated triarylmethane dyes) ^a	2.1×10^{-4} (50 th percentile)	2.2×10^{-5}
Consumer uses (Brilliant Blue FCF)	4.0×10^{-5} (50 th percentile)	4.3×10^{-6}

N/A = Not applicable

^a Non-sulfonated triarylmethane dyes = Basic Violet 3, Malachite Green, Basic Violet 4, and Basic Blue 7

^b A drinking water intake rate of 0.8 L/day and a body weight of 7.5 kg were used (Health Canada 1998).

To estimate potential cancer risk from daily exposure of Basic Violet 3, a lifetime-adjusted daily dose (LADD) was calculated (Appendix C). Drinking water intake rates and body weights from Health Canada (1998) were used for formula-fed infants, toddlers, children, teenagers and adults. The LADD for daily intake of Basic Violet 3 from drinking water was estimated to be 2.3×10^{-5} mg/kg bw/day (for the paper deinking scenario, as it represents the highest exposure). As other exposures were identified for Malachite Green, Basic Violet 4, Basic Blue 7 and Brilliant Blue FCF that were higher than those from environmental media, LADD were not calculated for those substances.

For Pigment Blue 61, due to a combination of its limited commercial quantities in Canada, negligible volatility, very low water solubility, and expected removal by water treatment systems, exposure from environmental media is not expected.

Food

With the exception of Brilliant Blue FCF, substances in the Triarylmethanes Group were not reported to be in food. JECFA has reported that Basic Violet 3 and Malachite Green

may be present in animal by-products/meats and/or fish as residues from continued use in veterinary drugs and aquaculture (EFSA 2016; JECFA 2009, 2014). Neither of these substances is currently approved for use in food-producing animals or fish in Canada (CFIA 2015; Health Canada [modified 2010]). Exposure of the general population to trace amounts of these substances in food due to non-compliant use or as a result of imported fish was determined to not pose a safety concern to consumers (CFIA 2012; Personal communication, email from the Risk Management Bureau, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, 2016; unreferenced). All six substances are potentially used in food packaging materials in contact with food. Brilliant Blue FCF may also be a component in incidental additives (e.g., cleaners and dish detergents) used in food processing plants. Exposure to these substances due to the presence in food packaging and/or as a component of an incidental additive (e.g., due to the food manufacturing process) is expected to be negligible (Personal communication, emails from the Food Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, 2016; unreferenced).

In Canada, Brilliant Blue FCF is permitted for use as a food additive in a number of foods at a maximum level of use of 100 ppm, singly, or in combination with Fast Green FCF in accordance with Item 4 of the *List of Permitted Food Colouring Agents* (Health Canada [modified 2015a]). It is also permitted for use in feta cheese at a maximum level of 0.10 ppm and in lumpfish caviar at a maximum level of 450 ppm in accordance with Items 4 and 9 of the *List of Permitted Food Colouring Agents* respectively (Health Canada [modified 2015a]). Dietary exposure to Brilliant Blue FCF was estimated using the levels measured by the Canadian Food Inspection Agency from its targeted surveys on food colours (CFIA 2010, 2011) and one-day recall food consumption data from the Canadian Community Health Survey Cycle 2.2 on Nutrition (Statistics Canada 2004). Where data were not available for a particular food category, the level of Brilliant Blue FCF measured in a similarly coloured food was applied. At the 90th percentile, the highest estimated dietary exposure of Brilliant Blue FCF as a result of its use as a food additive, on a body weight basis, was for children aged 4 to 8 years, which was 330 µg/kg bw/day (Personal communication, emails from the Food Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada, 2016-2017; unreferenced).

Based on consideration of the above information, the potential exposure of the general population to Brilliant Blue FCF through the dietary intake of food and drinking water is expected to be significantly lower than potential exposure through use of products available to consumers. Exposure of the remaining five substances of the Triarylmethanes Group through food and food packaging in Canada is expected to be minimal to negligible.

Products available to consumers

Exposures from the use of products available to consumers containing substances in the Triarylmethanes Group were evaluated. Product scenarios that result in the highest

levels of potential exposure for each substance by the oral, dermal, and inhalation routes are presented in Table 8-2, Table 8-3, and Table 8-4, respectively. Potential exposures were estimated based on conservative assumptions and using default values from sentinel exposure scenarios; see Appendix C for further details.

To estimate potential cancer risk, daily systemic exposures on an age group-specific basis were used except where lifetime adjustment was undertaken (see section 8.3 Characterization of Risk to Human Health). For those scenarios where such an adjustment was required, lifetime average daily doses (LADDs) were calculated.

Table 8-2. Estimated oral exposures to the Triarylmethanes Group from the use of products available to consumers

Substance	Product scenario	Age group	Per event systemic exposure (mg/kg bw)	Daily systemic exposure (mg/kg bw/day)
Malachite Green	Paint marker	Toddler	0.16	0.0081
Malachite Green	Paint marker	Child	0.081	0.0040
Brilliant Blue FCF	Non-medicinal use in a natural health product	Adult	NA	2.82

Abbreviation: NA: not applicable

Basic Violet 3's reported use as or in a pigment in paper products, mixtures, or manufactured items is limited to commercial applications in Canada. As such, potential oral exposure to this substance from products used by consumers is not expected. Similarly, based on its reported use as a pigment in printing ink limited to commercial applications in Canada, potential oral exposure to Pigment Blue 61 from products used by consumers is also not expected.

For estimated potential exposures via the dermal route, the maximum flux (J_{max}) approach (Williams et al. 2016) was used for Malachite Green, Basic Violet 4, and Basic Blue 7 to characterize systemic doses as a refinement (See Appendix C). A dermal flux study conducted by Lucová et al. (2013) showed the skin penetration of Brilliant Blue FCF in an oil-in-water emulsion to be practically negligible. In this study, the cumulative amount of Brilliant Blue FCF (oil-in-water emulsion) absorbed into the epidermis applied to an *ex vivo* sample of intact full thickness porcine ear skin (1 ± 0.07 mm) at a dermal load of 250 ng/cm² over 24 hours is 14 ng/cm² (with standard deviation, or SD, of 3 ng/cm²). Any amount of Brilliant Blue FCF that may have been present in the receptor fluid or the dermis was below the limit of quantitation. Since the stratum corneum was not separated from the viable epidermis, the total amount absorbed into the epidermis

of 17 ng/cm² (14 ng/cm² plus 1 SD) was conservatively used to estimate dermal absorption of Brilliant Blue FCF in this assessment. An *in vitro* percutaneous absorption study on Brilliant Blue FCF was also available (SCCNFP 2004), which similarly showed no measurable permeation through skin (i.e., in the receptor fluid) but conservatively estimated a maximum potential absorption of 6.2 µg/cm² for a hair colour gel formulation and 35.2 µg/cm² for the pure dye based on the limit of detection and skin-bound residues. Based on formulation considerations, the study by Lucová et al. (2013) was deemed more relevant to the body cream scenario, and was used accordingly.

Table 8-3. Estimated dermal exposures to the Triarylmethanes Group from the use of products available to consumers on an age group-specific basis

Substance	Product scenario	Age group	Per event systemic exposure (mg/kg bw)	Daily systemic exposure (mg/kg bw/day)
Malachite Green	Paint marker	Toddler	0.101	0.0051
Malachite Green	Paint marker	Child	0.051	0.0025
Malachite Green	Hair dye (semi-permanent)	Teenager	0.337	0.0033
Malachite Green	Hair dye (semi-permanent)	Adult	0.282	0.0102
Basic Violet 4	Hair dye (semi-permanent)	Teenager	0.0589	0.00058
Basic Violet 4	Hair dye (semi-permanent)	Adult	0.0494	0.00178
Basic Blue 7	Hair dye (semi-permanent)	Teenager	0.0177	0.000175
Basic Blue 7	Hair dye (semi-permanent)	Adult	0.0148	0.000533
Brilliant Blue FCF	Body cream	Infant	NA	0.0068
Brilliant Blue FCF	Body cream	Adult	NA	0.0041

Abbreviation: NA: not applicable

To estimate the potential cancer risk to intermittent per event dermal exposure, a lifetime average daily dose (LADD) of 0.00134 mg/kg bw/day was calculated for Basic Violet 4 for the use of hair dye by teenagers and adults. For all other dermal exposures

scenarios where potential cancer risks were estimated, the daily systemic exposures on an age group-specific basis were used (see section 8.3 Characterization of Risk to Human Health).

Although dermal contact with Pigment Blue 61 from printing ink is possible, the solubility of this pigment is very low and hence it will not readily solubilize in perspiration (BfR 2007). As a result, it cannot readily penetrate intact skin and therefore systemic exposure is not expected. Similarly, potential dermal contact with Basic Violet 3 based on its reported use as or in a pigment in paper products, mixtures or manufactured items is not expected to result in systemic exposure; migration of the substance from paper as a pigment component of an ink (e.g., printing ink) and subsequent absorption by intact skin is not expected. Pigments used in printing inks are frequently dyes (e.g., Basic Violet 3) rendered insoluble by complexing with a metal ion (IARC 1996). Further, systemic exposure to Basic Violet 3 would also not be expected from use as a paper dye because dyes contained within the matrix of the paper would be anticipated to exhibit minimal migration in a dermal scenario.

The estimated exposure to Brilliant Blue FCF in a perfume spray is presented below.

Table 8-4. Estimated inhalation exposures to the Triarylmethanes Group from the use of products available to consumers

Substance	Product scenario	Age group	Per event systemic exposure (mg/kg bw)	Daily systemic exposure (mg/kg bw/day)
Brilliant Blue FCF	Perfume	Teenager	NA	0.00064
Brilliant Blue FCF	Perfume	Adult	NA	0.00054

Due to the very low vapour pressures (10^{-13} mmHg or lower at 25°C) of all six members of the Triarylmethanes Group, any potential non-aerosol exposures via the inhalation route were considered to be limited.

8.2 Health effects assessment

There were limited chemical-specific hazard data for some substances in the Triarylmethanes Group. Analogues were considered based on similarities in their physical and chemical properties, metabolism, and structure. The chemical-specific data will be presented first, followed by analogue data used to inform the health effects characterization of substances in the Triarylmethanes Group.

Basic Violet 3

Basic Violet 3 toxicity was reviewed by JECFA (2014) and Diamante et al. (2009). Basic Violet 3 was classified as ECHA Globally Harmonized System Carc. 2 (or 1B if there is

more or equal to 0.1% of Michler's ketone) (EU 2008). In rats or mice a single or one-week daily gavage exposure of 4 up to 7 mg/kg bw(/day) Basic Violet 3 was rapidly but poorly absorbed, then distributed primarily to fat, liver, kidneys, and to a lesser extent to muscle, ovaries and testes (McDonald et al. 1984; McDonald 1989). It is metabolized through reduction and demethylation pathways, with primarily reduced metabolites such as leucogentian violet identified in tissues (McDonald and Cerniglia 1984; McDonald 1989). Basic Violet 3 was mostly (66-73%) excreted in faeces, and to a lesser extent in urine (less than 8%) (McDonald et al. 1984; McDonald 1989; Docampo and Moreno 1990). The developmental toxicity of Basic Violet 3 was assessed in both rabbits and rats. In rabbits gavaged gestation days (GDs) 6 to 19 with 0, 0.5, 1, or 2 mg/kg bw/day gentian violet, and sacrificed GD 30, at the lowest-observed-adverse-effect-level (LOAEL) of 0.5 mg/kg bw/day, there was both decreased fetal body weight and maternal toxicity (increased mortality, decreased body weight and body weight gain, clinical signs such as wheezing, diarrhea, and congestion) (NTP 1983). No no-adverse-effect-level (NOAEL) was determined since this was the lowest tested dose (JECFA, 2014). In comparison, no effects were observed at the lowest tested dose of 2.5 mg/kg bw/day in rats gavaged GDs 6 to 15 and sacrificed GD 20 (NTP 1982). Maternal toxicity (clinical signs of toxicity and decreased body weight gain) was only observed at 5 mg/kg bw/day, with developmental toxicity (increased incidences of short 13th rib, hydronephrosis, and hydroureter) observed at 10 mg/kg bw/day (NTP 1982).

No reproductive effects were observed up to the highest tested dose of 30 mg/kg bw/day Basic Violet 3 by diet in a 3-generation reproductive toxicity study in rats (Littlefield 1988). Parents were exposed to 0, 5, 15, or 30 mg/kg bw/day for 80 days pre-mating exposure, then mated twice to make F1a and b litters. F1a rats (2/sex/litter) were used for the chronic carcinogenicity study (Littlefield et al. 1989). Then F1b rats (1/sex/litter) were mated after 100-140 days to generate F2a litters, this mating was repeated to make F2b litters, which then similarly reproduced F3a litters. The parental NOAEL was 15 mg/kg bw/day based on decreased body weight at 30 mg/kg bw/day (Littlefield 1988). There was no NOAEL for offspring due to increased focal dilatation of the renal cortex and tubules and necrosis of the thymus in F3a weanlings (2/sex/litter) at the LOAEL of 5 mg/kg bw/day and above (Littlefield 1988). In 13 week dietary studies, there were also slight decreased body weight in rats fed 25 mg/kg bw/day and increased liver weight in dogs fed up to 16 mg/kg bw/day, both of which were the highest tested doses (Littlefield et al. 1989).

There were mixed results for the genotoxicity of Basic Violet 3, but overall it was considered genotoxic *in vitro*, based on positive cell mutation (Aidoo et al. 1990), chromosome aberration (Au et al. 1978, Au and Hsu 1979) and DNA binding and repair assays (Müller and Gautier 1975, Wakelin et al. 1981) reviewed in Mani and Bharagava (2016). In contrast, Basic Violet 3 did not affect the incidence of chromosomal aberrations in mice exposed via drinking water up to 8 mg/kg bw/day for one month, nor did up to 6 mg/kg bw intravenously for one hour damage spleen lymphocyte DNA in mice (Aidoo et al. 1990; Au and Hsu 1979). In a 2-year carcinogenicity study in mice fed approximately 0, 11/14, 32/36, and 64/71 mg/kg bw/day (males/females) Basic Violet 3, increased erythropoiesis in the spleen and atrophy of ovaries in females were observed

at the lowest tested dose of 14.3 mg/kg bw/day (Littlefield 1984; Littlefield et al. 1985). At approximately 36 mg/kg bw/day and above there were increased liver adenomas and carcinomas in females, both of which were also increased in males at 64 mg/kg bw/day, along with increased mortality in females. In a 2-year carcinogenicity study in rats, no adverse effects were observed at the lowest tested dose of 30/40 mg/kg bw/day (males/females) Basic Violet 3 by diet. At the LOAEL of 80/100 mg/kg bw/day (males/females), there was increased mortality (males) and increased follicular cell adenoma of the thyroid gland (females), as well as increased hepatocellular adenoma in both sexes of rats (Littlefield et al. 1989).

The incidence of increased hepatocellular carcinomas in female mice at 2-years was 7/185, 5/93, 30/93, or 73/95 for 0, 14, 36, or 71 mg/kg bw/day, respectively. The dose-response relationship for the two tumour types was similar (the incidence of hepatocellular adenomas in female mice at 2-years was 8/185, 8/93, 36/93, 20/95 for 0, 14, 36, or 71 mg/kg bw/day, respectively) (JECFA 2014). To determine the cancer risk to increased liver hepatocellular carcinomas, JECFA (2014) used US EPA BMDS version 2.2 to establish that use of the multistage model had an acceptable fit, had the lowest Akaike's Information Criterion value, and had the lowest benchmark dose value for 10% extra risk (19.9 mg/kg bw/day) and benchmark dose lower 95% confidence limit for 10% extra risk (BMDL10 of 16.8 mg/kg bw/day) among the models.

Malachite Green

Malachite Green was reviewed as part of an NTP carcinogenicity study (2005) and by EFSA (2016). When rats or mice were fed 5 or 30 mg/kg bw/day Malachite Green for 1 month, it remained mostly unmetabolized in the liver, but reduced to leucomalachite green, and to a lesser extent to mono- and di-desmethyl malachite green or mono- and di-desmethylleucomalachite green (Culp et al. 1999). Malachite Green Oxalate was considered an analogue of Malachite Green based on physical chemical and structural similarities (e.g., the structure of Malachite Green Oxalate is identical to that of Malachite Green except that the anionic moiety of the salt is oxalate instead of chloride). In a developmental study rabbits gavaged with 0, 5, 10, or 20 mg/kg bw/day Malachite Green Oxalate GDs 6 to 18 and sacrificed GD 30, the maternal NOAEL was 5 mg/kg bw/day (Meyer and Jorgenson 1983). At 10 mg/kg bw/day, there was decreased food consumption, body weight and body weight gain in dams. At 5 mg/kg bw/day and above there were increased pre-implantation losses, increased early fetal resorptions, decreased fetal survival, decreased fetal body weight, and increased skeletal deviations. Although there were limitations in this developmental study in rabbits with Malachite Green Oxalate, including an unknown number of developmental incidences per litter, and a poor dose-response in fetal effects, adverse developmental effects were observed at the lowest dose tested. On the basis of this study, Malachite Green is expected to have developmental effects at 5 mg/kg bw/day, based on increased pre-implantation losses, increased early fetal resorptions, decreased fetal survival, decreased fetal body weight, and increased skeletal deviations at the lowest tested dose of 5 mg/kg bw/day.

Malachite Green was not genotoxic *in vitro* in cell mutation assays, chromosomal aberration, and Comet assays (Stammati et al. 2005; Fessard 1999, Ferguson and Baguley 1988; Au and Hsu 1979; NTP 2004; Panandiker et al. 1994; Bose 2005). On the other hand it also inhibited polymerase I catalyzed DNA replication, induced single strand DNA breaks, and was cytotoxic *in vitro* (Wolfe 1977; Panandiker et al. 1994; Stammati et al. 2005). There was mixed evidence of genotoxicity *in vivo*, with negative micronucleus, *Hprt* or *cII* mutant assays in mice fed 0 or 43 mg/kg bw/day for four or six weeks (Mittelstaedt et al. 2004), but evidence of chromosomal aberrations, DNA fragmentation, sister chromatid exchange, and DNA adduct formation in mice or rats gavaged or fed for four weeks with approximately 4 to 78 mg/kg bw/day Malachite Green (Donya et al. 2012; Culp et al. 1999; Kasem et al. 2016).

In a 2-year dietary carcinogenicity study in female mice, no systemic toxicity or tumours were observed up to the highest tested dose of 67 mg/kg bw/day Malachite Green (NTP 2005). In contrast, female rats fed 0, 7, 21, or 43 mg/kg bw/day Malachite Green for 2 years had a NOAEL of 7 mg/kg bw/day, based on 9% decreased body weight and slightly increased tumour incidences at the LOAEL of 21 mg/kg bw/day and above (NTP 2005). Thyroid follicular cell adenoma and carcinomas [combined incidence: 0/46, 0/48, 3/47 (6%), 2/46 for control to high doses, respectively, historical control range up to 3%], and hepatocellular adenomas [1/48, 1/48, 3/48 (6%), 4/48 for control to high doses, respectively, historical control range up to 0.6%] were both supported by slight non-neoplastic changes (cystic follicles in thyroid gland, eosinophilic foci in liver) at the LOAEL of 21 mg/kg bw/day (NTP 2005). At 43 mg/kg bw/day there was a slight increase in mammary gland carcinomas [2/48, 2/48, 1/48, 5/48 (10%) for control to high doses, respectively, historical control range up to 4%] and increased relative liver weight in female rats. There were no thyroid hormone changes observed in a satellite 21 day study in rats fed 60 mg/kg bw/day Malachite Green (NTP 2005). In a four month oral study in rats, increased hepatocellular eosinophilic foci with 13 mg/kg bw/day Malachite Green in drinking water were transformed with diethylnitrosamine initiation into hepatocellular carcinomas accompanied by increased relative liver weight and cell cycle changes (Sundarrajan et al. 2000). NTP consider there to be equivocal evidence of carcinogenicity in female rats based on increased combined thyroid tumours, and marginal increases in hepatocellular adenoma and mammary gland carcinomas.

Pigment Blue 61

Pigment Blue 61 was not identified as posing a high hazard to human health on the basis of classifications by other national or international agencies for carcinogenicity, genotoxicity, developmental toxicity, or reproductive toxicity. It is also not on the European Chemicals Agency's Candidate List of Substances of Very High Concern for Authorisation (ECHA [modified 2016]). Further investigation of health effects is not warranted at this time given the low expected exposure of the general Canadian population.

Basic Violet 4

Basic Violet 4 was not genotoxic *in vitro* [bacterial reverse mutation assays, mammalian gene mutation assays (Chinese hamster ovary cells), and a mouse lymphoma assay (Seifried et al. 2006; ECHA c2007-2017b)]. The toxicity of Basic Violet 4 was informed by its analogue Ethyl Violet Acetate (CAS RN 63157-72-2), which was reviewed by ECHA (2015). Similar to the comparison of Malachite Green and its analogue Malachite Green Oxalate, the structure of Ethyl Violet Acetate is identical to that of Basic Violet 4 except that the anionic moiety of the salt is acetate instead of chloride. When no Ethyl Violet Acetate data were available, Basic Violet 3 was also used as an analogue, since it is also similar to Basic Violet 4 with respect to physical chemical properties and chemical structure.

In a developmental study with Ethyl Violet Acetate, there was no maternal or developmental effect up to the highest tested dose of 12 mg/kg bw/day in rats (ECHA c2007-2017b). This was consistent with a four-week gavage study in rats with Ethyl Violet Acetate, in which there were no adverse effects at 10 mg/kg bw/day, but there was a steep dose-response curve since severe toxicity was observed in both sexes at 20 to 30 mg/kg bw/day (including mortality, decreased body weight and body weight gain, decreased food consumption, and poor general condition) (ECHA c2007-2015b).

On the basis of a 3-generation reproductive toxicity study in rats exposed to 0, 5, 15, or 30 mg/kg bw/day Basic Violet 3 by diet (Littlefield 1988), Basic Violet 4 may result in offspring effects (focal dilatation of the renal cortex and tubules and necrosis of the thymus in F3a weanlings) and reduced maternal body weight gain at 5 mg/kg bw/day, but no reproductive effect is expected.

Similarly, based on Basic Violet 3, up to 6 or 8 mg/kg bw(/day) Basic Violet 4 was unlikely to increase chromosomal aberrations or damage DNA in mice (Aidoo et al. 1990; Au 1979; Diamante et al. 2009, Littlefield et al. 1985). A two-year carcinogenicity study in mice exposed to dietary Basic Violet 3 indicates that potential effects may be increased erythropoiesis in the spleen and atrophy of the ovaries in females at 14 mg/kg bw/day and above, and increased hepatocellular tumors at 36 mg/kg bw/day and above, with a BMDL10 of 16.8 mg/kg bw/day for increased risk of hepatocellular carcinomas in female mice (Littlefield 1984; Littlefield et al. 1985; JECFA 2014). In addition to similarities in the chemical structure and physical chemical properties of Basic Violet 3 and Basic Violet 4, the applicability of Basic Violet 3 data to characterize the carcinogenic potential of Basic Violet 4 was supported by similarities in their chemical profiles and (Q)SAR model predictions (Derek Nexus 2016; Leadscope Model Applier 2015; OECD QSAR Toolbox 2013; Times 2016). Further, metabolic pathways predicted by Times 2016 indicate that Basic Violet 4 may undergo the same metabolic transformations as Basic Violet 3 (Docampo and Moreno 1990), which further supports the read-across from Basic Violet 3 to Basic Violet 4 in the absence of a substance-specific carcinogenicity study.

Basic Blue 7

In the absence of any substance-specific hazard data, the toxicity of Basic Blue 7 was based upon analogues with physical chemical and structural similarities, with Basic Violet 4 and Ethyl Violet Acetate being more similar to Basic Blue 7 than Basic Blue 26 or Basic Violet 3. On the basis of a four-week study and a developmental study in rats gavaged with Ethyl Violet Acetate, up to 12 mg/kg bw/day Basic Blue 7 by gavage is not expected to result in any adverse developmental effect, with systemic toxicity including mortality expected at 20 mg/kg bw/day and above (ECHA c2007-2017). Up to 30 mg/kg bw/day Basic Blue 7 by diet is not expected to result in reproductive effects, based on a 3-generation study with Basic Violet 3 in rats (Littlefield 1988; JECFA 2014). The *in vitro* and *in vivo* genotoxicity of Basic Blue 7 is expected to be negative, in consideration of negative *in vitro* genotoxicity of Basic Violet 4 (Seifried et al. 2006; ECHA c2007-2017b), Basic Blue 26 (Janik-Spiechowicz et al. 1997; Nagai 1959) and negative *in vivo* genotoxicity of Basic Violet 3 or Basic Blue 26 (Janik-Spiechowicz et al. 1997). On the basis of a two-year carcinogenicity study in mice exposed to dietary Basic Violet 3, Basic Blue 7 is expected to increase erythropoiesis in the spleen and atrophy of the ovaries in females at 14 mg/kg bw/day and above, and increase the incidence of hepatocellular tumors at 36 mg/kg bw/day and above, with a BMDL10 of 16.8 mg/kg bw/day for increased risk of hepatocellular carcinomas in female mice (Littlefield 1984; Littlefield et al. 1985; JECFA 2014). In addition to similarities in the chemical structure and physical chemical properties of Basic Violet 3 and Basic Blue 7, the applicability of Basic Violet 3 data to characterize the carcinogenic potential of Basic Blue 7 was supported by similarities in their chemical profiles and (Q)SAR model predictions (Derek Nexus 2016; Leadscope Model Applier 2015; OECD QSAR Toolbox 2013; Times 2016). As in the case of Basic Violet 4, the metabolic pathways predicted by Times 2016 indicate that Basic Blue 7 may undergo the same metabolic transformations as Basic Violet 3 (Docampo and Moreno 1990), which further supports the read-across from Basic Violet 3 to Basic Blue 7 in the absence of a substance-specific carcinogenicity study.

Brilliant Blue FCF

Brilliant Blue FCF was reviewed by JECFA (1969), EFSA (2010), and US EPA (2013). It was poorly absorbed orally (2% in bile-duct ligated female rats), and excreted almost entirely (95.5 to 99.99%) as the parent compound in feces within three days (Brown et al. 1980; Phillips et al. 1980; EFSA 2010). There were two *in vitro* dermal absorption studies with Brilliant Blue FCF, as discussed in section 8.2 (SCCNFP 2004; Lucová et al. 2013). No adverse effects were observed after 75 weeks by diet in rats, establishing a NOAEL of 1500 mg/kg bw/day at the highest tested dose (Mannell et al. 1962; US EPA 2013). It was not mutagenic nor did it induce DNA damage *in vitro* (Borzelleca et al. 1990) nor micronuclei or DNA damage in mice *in vivo* (EFSA 2010). Positive *in vitro* genotoxicity results were attributed to purity of test materials (Borzelleca et al. 1990), which may also account for increased micronuclei in human lymphocytes with Brilliant Blue FCF *in vitro* (Kus and Eroglu 2015).

In a two-year dietary study with a reproductive study and *in utero* phase in rats, there was decreased terminal mean body weight and survival in females at the LOAEL of

1318 mg/kg bw/day, and a NOAEL of 631 mg/kg bw/day, with no reproductive effect (Borzelleca et al. 1990, EFSA 2010, US EPA 2013). The NOAEL for males was 1072 mg/kg bw/day. Consistent with such findings, no reproductive effects were observed in a dietary 3-generation reproductive toxicity study in rats (US EPA 2013), although there was decreased body weight [nursing offspring and F1 and F2 rats (detail regarding age not stated in EFSA review)] at the highest tested dose of 1000 mg/kg bw/day in this limited study) (Bio/dynamics Inc., 1972, 1973). In a reproductive/developmental study in mice offspring there were no adverse effects at 347 to 1287 mg/kg bw/day in males and females, with potential indications of offspring neurotoxicity only evidenced at high doses of 1032 to 3856 mg/kg bw/day (decreased surface righting at postnatal day 4, decreased horizontal activity at 8 weeks, and increased spontaneous activity in females) (Tanaka et al. 2012).

No tumours were observed at up to the highest tested dose of 2500 mg/kg bw/day in 2-year dietary rat studies or 7354/8966 mg/kg bw/day (males/females) in mice which were limited by a lack of detail including protocol description) (Hansen et al. 1966; Wilhelm and Ivy 1953; Klinke 1955; US EPA 2013). It may affect the endocrine system based on Toxcast and Tox21data searches (JMPR 2016). No developmental effects were identified in rat or rabbit studies with Brilliant Blue FCF (Burnett et al. 1974) in studies limited in detail (EFSA 2010; US EPA 2013), nor was developmental neurotoxicity observed *in vitro* (Lau et al. 2006). On the basis of available information, health effects of concern were not identified for Brilliant Blue FCF.

8.3 Characterization of risk to human health

The points of departures selected for risk characterization are summarized in Appendix D. Points of departure. Tables 8-5 to 8-8 provide all the relevant exposure estimates and hazard points of departure (PODs) for the substances in the Triarylmethanes Group, as well as resultant margins of exposure. Oral studies are used as surrogates for dermal or inhalation studies, in the absence of route-specific hazard data. For the per event exposure scenarios (for Malachite Green, Basic Violet 4, and Basic Blue 7), a point of departure from a developmental study is considered relevant to both prenatal and postnatal young, since effects in prenatal young suggest sensitivity of the young.

For daily exposures to Malachite Green, taking into consideration that its genotoxicity profile is mixed, that in carcinogenicity studies no neoplasia was observed in female mice up to 67 mg/kg bw/day, and that the liver and thyroid neoplasia in female rats at the LOAEL of 21 mg/kg bw/day and above had only a slight or no dose-response in the presence of decreased body weight, cystic follicles in the thyroid gland, and eosinophilic foci in the liver, use of the LOAEL of 5 mg/kg bw/day from the developmental toxicity study was considered protective of these effects observed at higher levels of exposure.

Environmental media

Table 8-5. Relevant oral systemic exposure estimates and hazard PODs for Basic Violet 3, as well as margins of exposure, for determination of risk from environmental media

Exposure scenario	Systemic exposure (mg/kg bw /day)	Critical effect level (mg/kg bw /day)	Critical health effect endpoint	MOE
Drinking water, Daily, Basic Violet 3	0.00010	LOAEL 0.5 (LTD)	Maternal toxicity (increased mortality, decreased body weight and body weight gain, clinical signs) and decreased fetal body weight	5000
Drinking water, LADD, Basic Violet 3	0.000023	BMDL10 of 16.8	Increased hepatocellular carcinomas	730 000

Abbreviations: LTD = lowest tested dose

These margins of exposure are considered adequate to address uncertainties in the health effects and exposure databases for both non-cancer and cancer effects.

Products available to consumers

Table 8-6. Relevant oral systemic exposure estimates and hazard PODs for the Triarylmethanes Group, as well as margins of exposure, for determination of risk

Exposure scenario	Systemic exposure [mg/kg bw (/day)]	Critical effect level [mg/kg bw (/day)]	Critical health effect endpoint	MOE
Paint marker, Toddler & child, Daily, Malachite Green	0.0081 (toddler); 0.0040 (child)	LOAEL 5 (LTD)	Increased pre-implantation loss, increased early fetal resorptions, decreased fetal survival, decreased fetal body weight, and increased skeletal deviations	620 (toddler); 1250 (child)

Paint marker, Toddler & child, Per event, Malachite Green	0.16 (toddler); 0.081 (child)	LOAEL 5 (LTD)	Increased pre-implantation loss, increased early fetal resorptions, decreased fetal survival, decreased fetal body weight, and increased skeletal deviations	31 (toddler);62 (child)
Non-medicinal use in a natural health product, Adult, Daily, Brilliant Blue FCF	2.82	NOAEL 631	Decreased body weight and survival in females at 1318 mg/kg bw/day	220

Abbreviations: LTD = lowest tested dose

Other potential oral exposures to Brilliant Blue FCF including through lipstick or lip balms (toddlers, adults), cold or allergy medication (children or adults), and dietary intake through food ranged from 0.1 to 1 mg/kg bw (/day), with MOEs of 630 to 6300. These margins of exposure are considered adequate to address uncertainties in the health effects and exposure databases for non-cancer effects. Furthermore, the estimated oral exposures to Brilliant Blue FCF (including its use in a natural health product) are below the upper bound of the acceptable daily intake (ADI) of 6 mg/kg bw/day established by EFSA, and subsequently adopted by JECFA in 2017 at which time the previous upper bound of the ADI of 12.5 mg/kg bw/day was withdrawn, which is based on a 100-fold uncertainty factor applied to the NOAEL of 631 mg/kg bw/day (EFSA 2010, JECFA 2017). A qualitative risk assessment approach as used by the US EPA (2013) was also considered, since no adverse effects were observed in chronic dietary studies up to 2500 mg/kg bw/day in rats and above 7354 mg/kg bw/day in mice, nor in a 3-generation reproductive toxicity study in rats up to 1000 mg/kg bw/day. However, a quantitative risk assessment was conducted since these other studies were limited in detail, the LOAEL of 1318 mg/kg bw/day was based in part on decreased survival, and the point of departure was in agreement with EFSA (2010).

Table 8-7. Relevant systemic dermal exposure estimates and hazard PODs for the Triarylmethanes Group, as well as margins of exposure, for determination of risk

Exposure scenario	Systemic exposure [mg/kg bw (/day)]	Critical effect level [mg/kg bw (/day)]	Critical health effect endpoint	MOE
Paint marker, Toddler & child, Daily, Malachite Green	0.0051 (toddler); 0.0025 (child)	LOAEL 5 (LTD)	Increased pre-implantation loss, increased early fetal resorptions, decreased fetal survival, decreased fetal body weight, and increased skeletal deviations	980 (toddler);2000 (child)
Paint marker, Toddler & child, Per Event Malachite Green	0.101 (toddler); 0.051 (child)	LOAEL 5 (LTD)	Increased pre-implantation loss, increased early fetal resorptions, decreased fetal survival, decreased fetal body weight, and increased skeletal deviations	50 (toddler);98 (child)
Hair dye (semi-permanent), Teenager & adult, Daily, Malachite Green	0.0033 (teen); 0.0102 (adult)	LOAEL 5 (LTD)	Increased pre-implantation loss, increased early fetal resorptions, decreased fetal survival, decreased fetal body weight, and increased skeletal deviations	1500 (teen);490 (adult)
Hair dye (semi-permanent), Teenager & adult, Per	0.337 (teen); 0.282 (adult)	LOAEL 5 (LTD)	Increased pre-implantation loss, increased early fetal	15 (teen);18 (adult)

Event, Malachite Green			resorptions, decreased fetal survival, decreased fetal body weight, and increased skeletal deviations	
Hair dye (semi- permanent), Teenager & adult, Per Event, Basic Violet 4	0.0589 (teen); 0.0494 (adult)	NOAEL 12 (HTD)	No observed maternal or developmental effects	200 (teen);240 (adult)
Hair dye (semi- permanent), Teenager & adult, Per Event, Basic Blue 7	0.0177 (teen); 0.0148 (adult)	NOAEL 12 (HTD)	No observed maternal or developmental effects	680 (teen);810 (adult)
Body cream, Infant & adult, Daily, Brilliant Blue FCF	0.0068 (infant); 0.0041 (adult)	NOAEL 12.6 ^a	Decreased body weight and survival in females at 1318 mg/kg bw/day	1900 (infant);310 0 (adult)

Abbreviations: LTD = lowest tested dose, HTD = highest tested dose

^a NOAEL 631 mg/kg bw/day x 2% oral absorption (Brown et al. 1980), which estimates the internal dose at which the critical health effects were observed.

Table 8-8. Relevant systemic dermal exposure estimates and cancer PODs for the Triarylmethanes Group, as well as MOEs, for determination of risk

Exposure Scenario	Systemic Exposure (mg/kg bw /day)	Critical effect level (mg/kg bw /day)	Critical health effect endpoint	MOE
Hair dye (semi- permanent), LADD, Basic Violet 4	0.00134	BMDL10 16.8	Increased hepatocellular carcinomas	13 000
Hair dye (semi- permanent), Teenager & adult, Daily, Basic Blue 7	0.000175 (teen); 0.000533 (adult)	BMDL10 16.8	Increased hepatocellular carcinomas	96 000 (teen) ^a ;32 000 (adult) ^a

^a The MOEs presented are considered to be conservative, as the exposures have not been adjusted to lifetime average daily doses (LADDs). Such adjustments were not performed as the MOEs for each individual age group are considered adequate, and the adjustments would result in higher MOEs due to a presumed typical lack of use by younger age groups.

Inhalation risks from daily exposure to Brilliant Blue FCF in perfume (0.00054 or 0.00064 mg/kg bw/day, for adults or teenagers, respectively) would result in MOEs (1 200 000 and 990 000, respectively) to the NOAEL of 631 mg/kg bw/day. These margins of exposure are considered adequate to address uncertainties in the health effects and exposure databases for non-cancer effects.

The MOEs between critical systemic effects and the estimates of per event or daily oral exposure to Malachite Green ranged from 31 to 1250 (paint marker). The MOEs from dermal exposure to Malachite Green ranged from 50 to 2000 (paint marker), and 15 to 1500 (hair dye) for all age groups. These may be potentially inadequate to account for uncertainties in the health effects and exposure databases for non-cancer effects, which includes consideration that the LOAEL selected as the point of departure was the lowest tested dose.

Comparison of estimated systemic dermal and oral exposures with the range of critical effect levels results in MOEs as follows: Basic Violet 4 ranged from 200 to 240; Basic Blue 7 ranged from 680 to 810; and Brilliant Blue FCF ranged from 220 to 3100. The potential cancer risk from daily exposures to Basic Violet 4 or Basic Blue 7 resulted in MOEs ranging from 13 000 to 96 000. The MOE for inhalation risk of Brilliant Blue FCF was above 990 000. These MOEs are considered adequate to address uncertainties in the exposure and health effect databases for both non-cancer and cancer effects.

While exposures of the general population to Basic Violet 3, Basic Violet 4, and Basic Blue 7 are not of concern at current levels, these substances are considered to have a health effect of concern based on their potential carcinogenicity. Basic Violet 3 was classified as ECHA GHS Carc 2 (or 1B if there is more or equal to 0.1% of Michler's ketone) (EU 2008). Therefore, there may be a concern for human health if exposures were to increase.

8.4 Uncertainties in evaluation of risk to human health

The key sources of uncertainty are presented in the table below.

Table 8-9. Sources of uncertainty in the risk characterization

Key sources of uncertainty	Impact
No chemical-specific empirical dermal absorption data were available, with the exception of Brilliant Blue FCF.	+/-
Although market surveillance was not undertaken, it was considered that colourants reported in craft products in the United States would also be	+

Key sources of uncertainty	Impact
available to the general population in Canada (e.g., via Canadian online retailers, retail chains present in both the United States and Canada).	
There is a lack of Canadian monitoring data for triarylmethanes in ambient environmental media (e.g., surface water) or drinking water	+/-
There are no sub-chronic or chronic animal studies via the dermal or inhalation routes, and limited chronic animal studies via the oral route, for substances in the Triarylmethanes Group.	+/-
There are limited reproductive toxicity or developmental studies for substances in the Triarylmethanes Group; for example, no reproductive toxicity study for Malachite Green was identified.	+/-

+ = uncertainty with potential to cause over-estimation of exposure/risk; - = uncertainty with potential to cause under-estimation of exposure risk; +/- = unknown potential to cause over or under estimation of risk.

9. Conclusion

Considering all available lines of evidence presented in this draft screening assessment, there is risk of harm to the environment, from Basic Violet 3, Malachite Green, Basic Violet 4, and Basic Blue 7. It is proposed to conclude that Basic Violet 3, Malachite Green, Basic Violet 4, and Basic Blue 7 meet the criteria under paragraph 64(a) of CEPA as they are entering or may enter 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. However, it is proposed to conclude that Basic Violet 3, Malachite Green, Basic Violet 4, and Basic Blue 7 do not meet the criteria under paragraph 64(b) of CEPA as they are not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger to the environment on which life depends.

Considering all available lines of evidence presented in this draft screening assessment, there is low risk of harm to the environment from Brilliant Blue FCF and Pigment Blue 61. It is proposed to conclude that Brilliant Blue FCF and Pigment Blue 61 do not meet the criteria under paragraphs 64(a) or (b) of CEPA as they are 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.

On the basis of the information presented in this draft screening assessment, it is proposed to conclude that Malachite Green meets the criteria under paragraph 64(c) of CEPA as it is entering or may enter the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

On the basis of the information presented in this draft screening assessment, it is proposed to conclude that Basic Violet 3, Pigment Blue 61, Basic Violet 4, Basic Blue 7, and Brilliant Blue FCF do not meet the criteria under paragraph 64(c) of CEPA as they

are 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.

Therefore, it is proposed to conclude that Basic Violet 3, Malachite Green, Basic Violet 4, and Basic Blue 7 meet one or more of the criteria set out in section 64 of CEPA. It is proposed to conclude that Pigment Blue 61 and Brilliant Blue FCF do not meet any of the criteria set out in section 64 of CEPA.

It is also proposed that Basic Violet 3, Malachite Green, Basic Violet 4, and Basic Blue 7 meet the persistence criteria but not the bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations* of CEPA.

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Appendix A. Aquatic toxicity

Table A-1. Key aquatic toxicity studies considered in choosing a critical toxicity value for water

Common name	Test organism	Endpoint	Effect	Value (mg/L)	Reference
Basic Violet 3	Japanese rice fish (<i>Oryzias latipes</i>)	48-h LC ₅₀	Mortality	0.1	Tonogai et al. (1982)
Malachite Green	Rainbow trout (<i>Oncorhynchus mykiss</i>)	96-h LC ₅₀	Mortality	0.25	Bills et al. (1977)
Malachite Green	Bluegill (<i>Lepomis macrochirus</i>)	96-h LC ₅₀	Mortality	0.03	Bills et al. (1977)
Malachite Green	Brown trout (<i>Salmo trutta m. lacustris</i>)	96-h LC ₅₀	Mortality	0.22	Bills et al. (1977)
Malachite Green	Smallmouth bass (<i>Micropterus dolomieu</i>)	96-h LC ₅₀	Mortality	0.045	Bills et al. (1977)
Malachite Green	American eel (<i>Anguilla rostrate</i>)	96-h LC ₅₀	Mortality	0.27	Hinton and Eversole (1978)
Malachite Green	American eel (<i>Anguilla rostrate</i>)	96-h LC ₅₀	Mortality	2.86	Hinton and Eversole (1978)
Malachite Green	Japanese rice fish (<i>Oryzias latipes</i>)	48-h LC ₅₀	Mortality	0.32	MITI (1992)
Malachite Green	Bacteria (<i>Vibrio fischeri</i>)	30 minute EC ₅₀	Reduction in bioluminescence	0.031	Hernando et al. (2007)
Malachite Green	Rainbow trout (<i>Oncorhynchus mykiss</i>)	96-h LC ₅₀	Mortality	0.267	Van Heerden et al. (1995)
Malachite Green	Japanese rice fish (<i>Oryzias latipes</i>)	48-h LC ₅₀	Mortality	0.3	Tonogai et al. (1982)
Malachite Green	American eel (<i>Anguilla rostrate</i>)	96-h LC ₅₀	Mortality	0.54	Hinton and Eversole (1979)
Malachite Green	Zebrafish embryos (<i>Danio rerio</i>)	96-h LC ₅₀	Mortality	0.042	White et al. (2012)
Malachite Green	Zebrafish larvae (<i>Danio rerio</i>)	96-h LC ₅₀	Mortality	0.376	White et al. (2012)

Common name	Test organism	Endpoint	Effect	Value (mg/L)	Reference
Malachite Green oxalate	Zebrafish embryo (<i>Danio rerio</i>)	96-h LC ₅₀	Mortality	0.331	White et al. (2012)
Malachite Green oxalate	Zebrafish embryo (<i>Danio rerio</i>)	96-h LC ₅₀	Mortality	0.264	White et al. (2012)
Malachite Green	Water flea (<i>Daphnia magna</i>)	48-h EC ₅₀	Growth	0.77	Kanhare et al. (2014)
Pigment Blue 61	Fish	Acute	NA	<70	US EPA (2002)
Pigment Blue 61	Invertebrates	Acute	NA	<70	US EPA (2002)
Pigment Blue 61	Algae	Acute	NA	<10	US EPA (2002)
Pigment Blue 61	Fish	Chronic	NA	<7	US EPA (2002)
Pigment Blue 61	Invertebrates	Chronic	NA	<7	US EPA (2002)
Pigment Blue 61	Algae	Chronic	NA	<1	US EPA (2002)
Pigment Blue 61	<i>Daphnia magna</i>	48 h EC ₅₀	Immobilization	>0.048	CPMA (2016)
Pigment Blue 61	Algae (<i>Pseudokirchnerie lla subcapitata</i>)	72-h NOEC	Growth rate reduction	0.422	CPMA (2016)
Pigment Blue 61	Algae (<i>Pseudokirchnerie lla subcapitata</i>)	72-h NOEC	Yield inhibition	<0.422	CPMA (2016)
Pigment blue 56 (analogue for Pigment Blue 61)	Zebrafish (<i>Brachydanio rerio</i>)	96-h LC ₅₀	Mortality	>500	CPMA (2016)
Brilliant Blue FCF	Japanese Rice fish (<i>Oryzias latipes</i>)	48-h LC ₀	Mortality	>3000	Tonogai et al. (1978)
Brilliant Blue FCF	<i>Daphnia sp.</i>	48-h LC ₅₀	Mortality	>1000	Federal Registry (1988)
Brilliant Blue FCF	Sewage bacteria	NA	Reduction of activity	>300	Ciba (1989)
Brilliant Blue FCF	Guppy (<i>Poecilia reticulata</i>)	48-h LC ₀	Mortality	>500	Ciba (1989)
Brilliant Blue FCF	Snail (<i>Indoplanorbis exustus</i>)	48-h LC ₅₀	Mortality	>100	Yasuhiro (1984)

Common name	Test organism	Endpoint	Effect	Value (mg/L)	Reference
Brilliant Blue FCF	<i>Daphnia carinata</i>	3-h LC ₅₀	Mortality	>100	Yasuhiro (1984)
Brilliant Blue FCF	Nagoya daruma pond frog (<i>Rana brevipoda porosa</i>)	48-h LC ₅₀	Mortality	>100	Yasuhiro (1984)
Brilliant Blue FCF	Japanese common toad (<i>Bufo bufo japonicus</i>)	48-h LC ₅₀	Mortality	>100	Yasuhiro (1984)
Brilliant Blue FCF	Common Carp (<i>Cyprinus carpio</i>)	48-h LC ₅₀	Mortality	>100	Yasuhiro (1984)
Brilliant Blue FCF	Coho Salmon (<i>Oncorhynchus kisutch</i>)	96-h LC ₅₀	Mortality	116	Wan et al. (1991)
Brilliant Blue FCF	Chinook salmon (<i>Oncorhynchus tshawytscha</i>)	96-h LC ₅₀	Mortality	185	Wan et al. (1991)
Brilliant Blue FCF	Chum salmon (<i>Oncorhynchus keta</i>)	96-h LC ₅₀	Mortality	213.5	Wan et al. (1991)
Brilliant Blue FCF	Pink salmon (<i>Oncorhynchus gorbuscha</i>)	96-h LC ₅₀	Mortality	119.5	Wan et al. (1991)
Brilliant Blue FCF	Rainbow trout (<i>Oncorhynchus mykiss</i>)	96-h LC ₅₀	Mortality	206	Wan et al. (1991)
Brilliant Blue FCF	Sockeye salmon (<i>Oncorhynchus nerka</i>)	96-h LC ₅₀	Mortality	90	Wan et al. (1991)

Abbreviations: NA: Not available; NOEC: No effect concentration; LCx: Lethal concentration for x% of the population; ECx: Effect concentration for x% of the population

Appendix B. Assumptions used in ecological exposure scenarios

Table B-1. Summary of assumptions for scenario 1: Paper dyeing

Variable name	Distribution type	Range of values	Units	Additional comments
Paper production capacity	N/A	CBI	T/day	ECCC compiled data
Usage rate of the chemical ^a	Uniform distribution	10–100	g/T	ECCC compiled data and section 71 survey data
Usage rate of the chemical ^a	Uniform distribution	3.1–31	g/T	ECCC compiled data
Usage rate of the chemical ^a	Uniform distribution	30–100	g/T	OECD's Emission Scenario Document #23
Fraction of the product containing substance	N/A	1.0	Fraction	Assumption. In a worst case scenario, assuming a facility uses the chemical to dye all its paper to some extent.
Fraction of the mills inputs that are from recycled paper	N/A	CBI	Fraction	ECCC compiled data
Fraction of the chemical (CAS) in the dye compound used	Beta distribution	0.10–1.0	Fraction	ECCC compiled data
Retention rate	Beta distribution	0–1.0	Fraction	ECCC compiled data
Removal rate of secondary treatment	Beta distribution	0–1	Fraction	SimpleTreat models were run for applicable removal rates for WWTS. (P10 = 0.82; P90 = 0.92)
Removal rate of lagoon treatment	Beta distribution	0–1	Fraction	STP-EX models were run for applicable removal rates for lagoon systems. (P10 = 0.84; P90 = 0.88)
Effluent flow rate	N/A	CBI	m ³ /s	ECCC compiled data
Flow rate for the receiving water body	N/A	CBI	m ³ /s	ECCC compiled data. 10th percentile flow rate is used.

Variable name	Distribution type	Range of values	Units	Additional comments
Dilution factor	N/A	Capped at 10	Unitless	Actual dilution factor is used when DF is below 10. Maximum DF of 10 is used when actual DF is greater than 10.

Abbreviations: CBI: confidential business information; DF: dilution factor; ECCC: Environment and Climate Change Canada; N/A: not applicable; P10: value at the 10th percentile of the distribution; P90: value at the 90th percentile of the distribution.

^a Equal probability of any one of the three references used for usage rate of the chemical.

Table B-2. Summary of assumptions for scenario 2: Paper de-inking

Variable name	Distribution type	Range of values	Units	Additional comments
Capacity of a facility to de-ink paper	N/A	CBI	T/day	ECCC compiled data
Fraction of paper that has been covered in ink	Uniform distribution	5–50	%	Assumptions
Amount of coverage that can be expected on a piece of paper for a given amount of Ink	Uniform distribution	150 000 – 350 000	in ² [paper] /lbs [ink]	Assumptions
Density of paper	Uniform distribution	75–105	g/m ²	ECCC compiled data
Concentration fraction of chemical in ink	Uniform distribution	0.0001–0.02	Fraction	ECCC compiled data
Fraction of ink with chemical of interest in it	Uniform distribution	0.01–1.0	Fraction	Assumptions
Emission factor	Uniform distribution	0.1–0.2	Fraction	ECCC compiled data
Effluent flow rate	N/A	CBI	m ³ /s	ECCC compiled data
Flow rate for the receiving water body	N/A	ECCC compiled data	m ³ /s	10th percentile flow rate is used.

Dilution factor	N/A	Capped at 10	unitless	Actual dilution factor is used when DF is below 10. Maximum DF of 10 is used when actual DF is greater than 10.
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Abbreviations: CBI: confidential business information; DF: dilution factor; ECCC: Environment and Climate Change Canada; N/A: not applicable.

Table B-3. Summary of assumptions for scenario 3: General formulation

Variable Name	Distribution Type	Range of Values	Units	Additional Comments
Mass of chemical processed at a facility	Lognormal distribution	CBI	kg/yr	ECCC compiled data
Emission factor	Uniform distribution	0.002–0.011	Fraction	ECCC compiled data
Days of release	N/A	250	days/yr	Standard value for large volume chemical and continuous use.
Effluent flow rate	N/A	CBI	m ³ /day	ECCC compiled data
Flow rate for the receiving water body	N/A	ECCC compiled data	m ³ /s	10th percentile flow rate is used.
Dilution factor	N/A	Capped at 10	unitless	Actual dilution factor is used when DF is below 10. Maximum DF of 10 is used when actual DF is greater than 10.

Abbreviations: CBI: confidential business information; DF: dilution factor; ECCC: Environment and Climate Change Canada; N/A: not applicable.

Appendix C. Estimated potential human exposures to triarylmethanes from products used by consumers

All assumptions (Table C-2) were ConsExpo default assumptions (ConsExpo 2006) unless otherwise noted. For dermal exposure estimates, an overall retention factor (RF) of 1 was used, unless otherwise specified. Inhalation absorption was assumed to be 100%.

Maximum Flux Approach

As a refinement, the maximum flux (Jmax) approach as conducted in Williams et al. (2016) was used to estimate dermal exposures for Malachite Green, Basic Violet 4 and Basic Blue 7.

The equations used are provided below. Values for water solubility, log Kow, and molecular weight (MW) were obtained from Table 3-1 of this screening assessment report (where available, experimental values were used). A mass balance check was also done for each scenario; see Table C-2 below.

Kp (Potts & Guy equation, based on aqueous vehicle):
Log Kp (in cm/h) = -2.71 + (0.71)(log Kow) - (0.0061)(MW, in g/mol)

Jmax:
Jmax (in mg/cm²/h) = Kp (in cm/h) x Water solubility (in mg/cm³)

Maximum theoretical amount absorbed per day (Qabs):
Qabs (in mg) = Jmax (in mg/cm²/h) x Surface area of skin contact (in cm²) x Exposure duration (in h)

Dermal Systemic Exposure = Qabs/BW

The resulting dermal systemic exposure estimate represents a “per event” estimate where exposure frequency “F” was < 1/day and would theoretically represent a “daily” estimate where “F” were to be ≥ 1/day. However, it should be noted that there are no exposure scenarios for Malachite Green, Basic Violet 4, or Basic Blue 7 that fall into the latter category. Amortization of a “per event” estimate to generate a daily systemic exposure estimate was performed where relevant.

A mass balance check was conducted by comparing the Qabs to the total amount of the substance on the skin (Qapp; which is referred to in Table C-2 as the “dermal load”)

For mass balance check:
Qapp = Conc x Product Amount x RF (see individual exposure scenarios in Table C-2 for specific values; note that F is not applicable in the mass balance check since there are no exposure scenarios where exposure frequency exceeds once per day)

If the $Q_{abs} > Q_{app}$, then Q_{app} (equivalent to 100% dermal absorption) was used to characterize the amount absorbed. Otherwise, Q_{abs} was used.

Table C-1. Dermal exposure parameters for maximum flux approach for Malachite Green, Basic Violet 4, and Basic Blue 7 (on a 'day of exposure' basis)^a

Substance and sentinel exposure scenario	Age group(s)	Jmax (mg/cm ² /h)	Qabs (mg)
Malachite Green, paint marker	Toddler, Child	0.0013	1.6 (per event exposure scenario), 0.078 (daily exposure scenario) ^b
Malachite Green, semi-permanent hair dye	Teen, Adult	0.0013	20
Basic Violet 4, semi-permanent hair dye	Teen, Adult	8.7×10^{-4}	13
Basic Blue 7, semi-permanent hair dye	Teen, Adult	0.072	1.1×10^3

^a See exposure scenarios in Table C-2 for frequency (F), if relevant.

^b See Table C-2 for details on the per event and daily exposure scenarios.

Lifetime average daily dose (LADD)

The LADD was calculated as a refinement for dermal exposure to semi-permanent hair dye exposures to Basic Violet 4 to account for use of this product by teenagers and adults, as well as to estimate the potential cancer risk from daily exposure to Basic Violet 3 from drinking water. The assumptions and equation are provided below:

DSE: daily systemic exposure

Average lifetime (AL): 70 years (US EPA 2011)

Age group durations (AD): 0.5 years for infants (0 to 0.5 years), 4.5 years for toddlers (0.5 to 4 years), 7 years for children (5 to 11 years), 8 years for teens (12 to 19 years) and 50 years for adults (20+ years) (Health Canada 1998)

$$LADD = \frac{[(DSE_{\text{infant}} \times AD_{\text{infant}}) + (DSE_{\text{toddler}} \times AD_{\text{toddler}}) + (DSE_{\text{child}} \times AD_{\text{child}}) + (DSE_{\text{teen}} \times AD_{\text{teen}}) + (DSE_{\text{adult}} \times AD_{\text{adult}})]}{[AL]}$$

Dermal exposures to Brilliant Blue FCF

The potential absorbable dose of Brilliant Blue FCF from the dermal flux study by Lucová et al. (2013) was used to characterize systemic exposures for each dermal scenario. The following parameters, algorithms and considerations were used.

AV: skin surface area exposed

PAA: potential absorbable dose (over 24 hours of exposure)

F: exposure frequency

Conc: concentration

RF: Retention factor

Per Event Systemic Exposure = $(AV \times PAA)/BW$

For mass balance check:

(Total) Dermal Load = $Conc \times Product\ Amount \times RF \times F$

(where "F" is only incorporated if >1)

If the per event systemic exposure was less than the (total) dermal load, the per event systemic exposure was used to characterize systemic exposure given the lack of full dose depletion, otherwise the (total) dermal load was used (due to full dose depletion). Where "F" is $>1/day$, the per event systemic exposure can be used as a daily systemic exposure estimate as "PAA" represents the cumulative amount absorbed over 24 hours.

Table C-2. Sentinel exposure scenario assumptions

Substance	Sentinel exposure scenario	Assumptions ^a
Malachite Green	Paint marker	<p>Concentration (Conc): $\leq 5\%$ (MSDS 2014) Age group: Toddler^b and Child^c Body weight (BW): 15.5 kg for Toddler and 31.0 kg for Child</p> <p>For Estimated Per Event Oral or Dermal Exposure: Product amount (Amt): 50 mg (amount of ink on a skin surface area equivalent to two child palms of 5 x 5 cm², Hansen et al. 2008)^{d,e} Surface area of skin contact (SA): 50 cm² (Hansen et al. 2008) Exposure duration: 24 h/day</p> <p>For Per Event Oral Exposure = $(Conc \times Amt)/(BW)$ For Per Event Dermal Exposure, see Jmax approach above (Williams et al. 2016)</p>

Substance	Sentinel exposure scenario	Assumptions ^a
		<p>For Estimated Daily Oral or Dermal Exposure: Daily ink line: 25 cm/day^{d,f} Ink laydown rate: 100 µg/cm^{d,f} Surface area of skin contact (SA): 2.5 cm² (assume same proportion of ink per cm² of skin as acute scenario, i.e., corresponding to 1 mg/cm²) Exposure duration: 24 h/day</p> <p>For Daily Oral Exposure = (Daily ink line x Ink laydown rate x Conc)/(BW) For Daily Dermal Exposure, see Jmax approach above (Williams et al. 2016)</p> <p>For mass balance check (Per Event and Daily Dermal Exposure): Dermal load = Conc x Product Amount</p>
Malachite Green, Basic Violet 4, and Basic Blue 7	Hair dye (semi-permanent)	<p>Concentration: ≤1% for Malachite Green, ≤0.1% for Basic Violet 4, and ≤0.03% for Basic Blue 7 (2016 email from Consumer Product Safety Directorate, Health Canada to Existing Substances Risk Assessment Bureau, Health Canada; unreferenced) Age group: Teenager and Adult Body weight (BW): 59.4 kg for Teenager and 70.9 kg for Adult Frequency (F): 0.0099/day for Teenager (Statistics Canada 2012)⁹ and 0.036/day for Adult (SCCS 2016) Product amount: 35 g/application (SCCS 2016) Retention factor (RF): 0.1 (SCCS 2016) Surface area of skin contact (SA): 637.5 cm² (Based on ½ surface area of adult head; Health Canada 1995) Exposure duration: 24 h/day</p> <p>For Per Event and Daily Dermal Exposure, see Jmax approach above (Williams et al. 2016)</p> <p>For mass balance check (Per Event and Daily Dermal Exposure): Dermal load = Conc x Product Amount x RF</p>
Brilliant Blue FCF	Non-medicinal use in a natural	Amount per dose (Amt): 200 mg/scoop (2017 email from Natural and Non-prescription Health Products Directorate, Health Canada to Existing Substances Risk Assessment Bureau, Health Canada; unreferenced)

Substance	Sentinel exposure scenario	Assumptions ^a
	health product	<p>Maximum Daily Dose (MDD) = 1 scoop (2017 email from Natural and Non-prescription Health Products Directorate, Health Canada to Existing Substances Risk Assessment Bureau, Health Canada; unreferenced)</p> <p>Age group: Adult Body weight (BW): 70.9 kg Frequency (F) = 1/day</p> <p>For Estimated Daily Oral Exposure: $= (\text{Amt} \times \text{MDD} \times \text{F}) / (\text{BW})$</p>
Brilliant Blue FCF	Body cream	<p>Concentration (Conc): $\leq 10\%$ (2016 email from Consumer Product Safety Directorate, Health Canada to Existing Substances Risk Assessment Bureau, Health Canada; unreferenced)</p> <p>Age group: Infant and Adult Body weight (BW): 7.5 kg for Infant and 70.9 kg for Adult</p> <p>For Estimated Daily Dermal Exposure: Surface area of skin contact (AV)^h: 3020 cm² for Infant and 16925 cm² for Adult (Health Canada 1995) Product amount per application = 1.4 g for Infant and 4.4 g for Adult (Wormuth et al. 2006; Loretz et al. 2005) PAA: 17 ng/cm² (Lucová et al. 2013) Frequency (F) = 1.7/day for Infant and 1.1/day for Adult (Wormuth et al. 2006; Loretz et al. 2005)</p> <p>See potential absorbable dose approach above</p>
Brilliant Blue FCF	Perfume (aerosol spray)	<p>Concentration: $\leq 30\%$ (2016 email from Consumer Product Safety Directorate, Health Canada to Existing Substances Risk Assessment Bureau, Health Canada; unreferenced)</p> <p>Age group: Teenager and Adult Body weight: 59.4 for Teenager and 70.9 kg for Adult</p> <p>For Estimated Daily Inhalation Exposure, default parameters for spray model, eau de toilette fragrance product (ConsExpo Web 2016)ⁱ unless noted otherwise: Frequency: 1.7/day (Loretz et al. 2006) Mode of release: Spraying (towards person)</p>

Substance	Sentinel exposure scenario	Assumptions ^a
		Spray duration: 0.08 min Exposure duration: 5 min Room volume: 10 m ³ Room height: 2.5 m Ventilation rate: 2h ⁻¹ Cloud volume: 0.0625 m ³ Mass generation rate: 0.1 g/s Airborne fraction: 0.02 g/g Density non-volatile: 1.5 g/cm ³ Median particle diameter: 2.7 µm Maximum particle diameter: 50 µm Inhalation cut-off diameter: 10 µm Inhalation rate (Adult; used for both Adult and Teenager as it is protective): 16.2 m ³ /day (Health Canada 1998)

^a The age ranges for an infant, a toddler, a child, a teenager, and an adult were assumed to be newborn to 6 months, 0.5 to 4 years, 5 to 11 years, 12 to 19 years, and 20 to 59 years, respectively. Default body weights were obtained from Health Canada (1998).

^b This age group is considered to be the most highly exposed through the use of markers or pens, particularly in terms of mouthing behaviours (US EPA 2008).

^c The particular product (MSDS 2014) appears to be marketed towards children older than toddlers (e.g., 5 years old and up). However, to be protective, the potential for non-target use by toddlers could not be precluded.

^d The consistency (i.e., density) of paint in paint pens (may also be referred to as paint markers) is estimated to be similar to that of marker ink, as opposed to acrylic paint that is applied with a brush. As such, exposure defaults for ink were considered to be an appropriate surrogate.

^e Per event exposure is representative of potential scenarios that could occur in one day (e.g., children painting on their skin), but would not necessarily be expected to occur on a daily basis. Due to this difference, the per event exposure estimates are higher than daily exposure estimates.

^f Ink laydown rate reported by the Art and Creative Materials Institute (ACMI), Duke University (Personal communication from ACMI to Existing Substances Risk Assessment Bureau, Health Canada, 2009; unreferenced). ACMI reported that an individual may be exposed to an estimated 25 cm of ink line per day, through skin contact or incidental mouthing.

^g Statistics Canada (2012) survey question referred to generic "Hair Dyes" and did not specify specific type (e.g., Permanent, Semi-Permanent, Temporary). Used dataset pertaining to 12 to 19 year old group for Semi-Permanent Hair Dyes (used median value).

^h Total body surface area minus surface of head.

ⁱ The default scenario for Application, Exposure to Spray - Spraying using the factsheet for Eau-de-toilette in ConsExpo Web (2016) was applied.

Appendix D. Points of departure for human health risk characterization

Substance	Per event exposure	Daily exposure (systemic)
Basic Violet 3	Developmental study (rabbits, gavage) Maternal toxicity (increased mortality, decreased body weight and body weight gain, clinical signs) and decreased fetal body weight at the LOAEL of 0.5 mg/kg bw/day (LTD)	Default to the per event point of departure. [The LOAEL (LTD) of 14.3 mg/kg bw/day (increased erythropoiesis in the spleen, atrophy of the ovaries) from a dietary carcinogenicity study in female mice would not be protective of potential effects at the LOAEL of 0.5 mg/kg bw/day.]
Malachite Green	Developmental study (rabbits, gavage, Malachite Green Oxalate) Increased pre-implantation losses and early fetal resorptions, decreased fetal survival, decreased fetal body weight, and increased skeletal deviations at the LOAEL of 5 mg/kg bw/day (LTD)	Default to the per event point of departure. [The NOAEL of 7 mg/kg bw/day from a dietary carcinogenicity study in rats would not be protective of potential developmental effects at the LOAEL of 5 mg/kg bw/day.]
Pigment Blue 61	NA	NA
Basic Violet 4	Developmental study (rats, gavage, Ethyl Violet Acetate) No observed maternal or developmental effects at the NOAEL of 12 mg/kg bw/day (HTD)	NA
Basic Blue 7	Developmental study (rats, gavage, Ethyl Violet Acetate) No observed maternal or developmental effects at the NOAEL of 12 mg/kg bw/day (HTD)	NA
Brilliant Blue FCF	NA	2 year study with a reproductive study and an <i>in utero</i> phase (rats, dietary) NOAEL of 631 mg/kg bw/day based on decreased body weight and survival in females at 1318 mg/kg bw/day.

Abbreviations: HTD = highest tested dose, LOAEL = lowest observed adverse effect level, LTD = lowest tested dose, NA = not applicable, NOAEL = No observed adverse effect level

The BMDL10 of 16.8 mg/kg bw/day was used to estimate the cancer risk for all dermal and oral daily exposure scenarios of Basic Violet 3, Basic Violet 4, and Basic Blue 7. It was based on increased hepatocellular carcinomas in female mice fed Basic Violet 3 in a carcinogenicity study.