

Screening Assessment for the Challenge

**2-Naphthalenol, 1-[(4-methyl-2-nitrophenyl)azo]-
(Pigment Red 3)**

**Chemical Abstracts Service Registry Number
2425-85-6**

**Environment Canada
Health Canada**

February 2009

Synopsis

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the Ministers of the Environment and of Health have conducted a screening assessment on 2-Naphthalenol, 1-[(4-methyl-2-nitrophenyl)azo]- (Pigment Red 3), Chemical Abstracts Service Registry Number 2425-85-6. This substance was identified as a high priority for screening assessment and included in the Challenge because it had been found to meet the ecological categorization criteria for persistence, bioaccumulation potential and inherent toxicity to non-human organisms, and is believed to be in commerce in Canada.

The substance Pigment Red 3 was also considered to be a priority for assessment of potential risks to human health, based on meeting the categorization criteria for greatest potential for exposure. Therefore, this assessment focuses on information relevant to the evaluation of both human health and ecological risks.

Pigment Red 3 is an organic azo pigment that is primarily used in Canada and elsewhere as a red pigment in paints. The substance is not naturally produced in the environment. In Canada, one company manufactured Pigment Red 3 in 2006 in a quantity ranging from 100 000 to 1 000 000 kg, of which all but 30 000 to 50 000 kg was exported from Canada. Seven companies reported importing a total of 10 000 to 100 000 kg into Canada. Nine companies reported using a total of approximately 40 000 kg of Pigment Red 3 in Canada. The quantity of Pigment Red 3 in commerce in Canada, along with the potentially dispersive uses of this substance, indicate that it could be released into the Canadian environment.

Based on reported use patterns and certain assumptions, most of the substance ends up in waste disposal sites. Small proportions are estimated to be released to water (4.7%), and soil (1.6%). Pigment Red 3 exists in the environment in particulate form, which is not soluble in water or volatile. For these reasons, Pigment Red 3 is likely to settle by gravity to sediments if released to water, and will tend to remain in soils if released to terrestrial environments. It is not expected to be significantly present in other media. It is also not expected to be subject to long-range atmospheric transport.

Based on its physical and chemical properties, Pigment Red 3 is persistent in water, soil and sediment. New experimental data relating to its solubility in water and octanol suggest that this pigment has a low potential to bioaccumulate in the lipid tissues of organisms. The substance therefore meets the persistence criterion but does not meet the bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations*. In addition, new experimental toxicity data, as well as new toxicity predictions that take into account revised estimates of bioaccumulation potential, suggest that the substance has negligible to low potential for toxicity to aquatic organisms.

For the ecological assessment, a very conservative exposure scenario was designed in which it is assumed that all industrial operations (manufacturers and users of the pigment) discharge Pigment Red 3 into the aquatic environment at a single discharge point. The predicted environmental concentration in water was below the predicted no-effect concentration calculated for sensitive aquatic organisms. Additionally, since Pigment Red 3 may be used in consumer products, a conservative consumer release scenario was also developed based on the quantity of Pigment Red 3 in commerce. This scenario predicted that all of the Canadian watercourses modelled would have predicted environmental concentrations below the predicted no-effect concentration.

Based on the information available, it is concluded that Pigment Red 3 is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity.

Based on consideration of relevant available information, including weight-of-evidence based assessments by international and national agencies, a critical effect for the characterization of risk to human health for Pigment Red 3 is carcinogenicity. The exposure-related tumours were observed at multiple sites in male and female rats and male mice. Although the mode of induction of tumours has not been elucidated, in light of the equivocal evidence for genotoxicity including some positive mutagenicity and DNA damage from a limited database, a mode of tumour induction involving direct interaction with genetic material cannot be precluded.

On the basis of the carcinogenicity of Pigment Red 3, for which there may be a probability of harm at any level of exposure, it is concluded that Pigment Red 3 is a substance that may be entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

This substance will be included in the upcoming *Domestic Substances List* inventory update initiative. In addition and where relevant, research and monitoring will support verification of assumptions used during the screening assessment and, where appropriate, the performance of potential control measures identified during the risk management phase.

Based on the information available, it is proposed that Pigment Red 3 meets one or more of the criteria set out in section 64 of CEPA 1999.

Introduction

The *Canadian Environmental Protection Act, 1999* (CEPA 1999) (Canada 1999) requires the Minister of the Environment and the Minister of Health to conduct screening assessments of substances that have met the categorization criteria set out in the Act to determine whether these substances present or may present a risk to the environment or human health. Based on the results of a screening assessment, the Ministers can propose to take no further action with respect to the substance, to add the substance to the Priority Substances List (PSL) for further assessment, or to recommend that the substance be added to the List of Toxic Substances in Schedule 1 of the Act and, where applicable, the implementation of virtual elimination.

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

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

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

The substance 2-naphthalenol, 1-[(4-methyl-2-nitrophenyl)azo]- was identified as a high priority for assessment of ecological risk as it had been found to be persistent, bioaccumulative and inherently toxic to aquatic organisms and is believed to be in commerce in Canada. The Challenge for this substance was published in the *Canada Gazette* on August 18, 2007 (Canada 2007a). A substance profile was released at the same time. The substance profile presented the technical information available prior to December 2005 that formed the basis for categorization of this substance. As a result of the Challenge, submissions of information pertaining to the properties, bioaccumulation potential, hazard and uses of the substance were received.

The substance 2-naphthalenol, 1-[(4-methyl-2-nitrophenyl)azo]- was also considered a priority for assessment of potential risks to human health, based on meeting the categorization criteria for greatest potential for exposure. Therefore, this assessment

focuses on information relevant to the evaluation of both human health and ecological risks.

Under CEPA 1999, screening assessments focus on information critical to determining whether a substance meets the criteria for defining a chemical as toxic as set out in section 64 of the Act, where

“64. [...] a substance is toxic if it is entering or may enter the environment in a quantity or concentration or under conditions that

- (a) have or may have an immediate or long-term harmful effect on the environment or its biological diversity;
- (b) constitute or may constitute a danger to the environment on which life depends; or
- (c) constitute or may constitute a danger in Canada to human life or health.”

Screening assessments examine scientific information and develop conclusions by incorporating a weight of evidence approach and precaution as required under CEPA 1999.

This screening assessment includes consideration of information on chemical properties, hazards, uses and exposure, including the additional information submitted under the Challenge. Data relevant to the screening assessment of this substance were identified in original literature, review and assessment documents, stakeholder research reports and from recent literature searches, up to July 2007 for ecological sections of the document. Key studies were critically evaluated; modelling results may have been used to reach conclusions. When available and relevant, information presented in hazard assessment from other jurisdictions was considered. Evaluation of risk to human health involves consideration of data relevant to estimation of exposure (non-occupational) of the general population, as well as information on health hazards (based principally on the weight of evidence assessments of other agencies that were used for prioritization of the substance). Decisions for human health are based on the nature of the critical effect and/or margins between conservative effect levels and estimates of exposure, taking into account confidence in the completeness of the identified databases on both exposure and effects, within a screening context. The screening assessment does not represent an exhaustive or critical review of all available data. Rather, it presents a summary of the critical information upon which the conclusion is based.

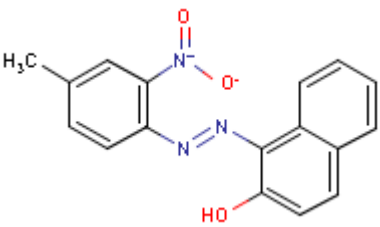
This screening assessment was prepared by staff in the Existing Substances Programs at Health Canada and Environment Canada and incorporates input from other programs within these departments. This assessment has undergone external written peer review/consultation on health section of the document. Comments on the technical portions relevant to human health were received from scientific experts selected and directed by Toxicology Excellence for Risk Assessment (TERA), including Dr. Donna J. Vorhees (The Science Collaborative North Shore), Dr. Susan Griffin (U.S. Environmental Protection Agency), and Joan Strawson (TERA). While external comments were taken into consideration, the final content and outcome of the screening risk assessment remain the responsibility of Health Canada and Environment Canada. Additionally, the draft of this screening assessment was subject to a 60-day public

comment period. While external comments were taken into consideration, the final content and outcome of the screening risk assessment remain the responsibility of Health Canada and Environment Canada. The critical information and considerations upon which the assessment is based are summarized below.

Substance Identity

For the purposes of this document, this substance will be referred to as Pigment Red 3.

Table 1. Substance identity for Pigment Red 3

Chemical Abstracts Service Registry Number (CAS RN)	2425-85-6
DSL name¹	2-Naphthalenol, 1-[(4-methyl-2-nitrophenyl)azo]-
National Chemical Inventories (NCI) names²	2-Naphthalenol, 1-[2-(4-methyl-2-nitrophenyl)diazenyl]-(TSCA) 2-Naphthalenol, 1-[(4-methyl-2-nitrophenyl)azo]- (AICS, PICCS, ASIA-PAC, NZIoC) 1-(4-methyl-2-nitrophenylazo)-2-naphthol (EINECS) Pigment Red 3 (ENCS) Pigment red 003 (ECL) 2-Naphthalenol, 1-[(4-methyl-2-nitrophenyl)azo]-(toluidine red) (PICCS) C.I. Pigment Red 3, Naphth-2-ol, 1-[(4-methyl-2-nitrophenyl)azo](Toluidine Red) (PICCS) Toluidine Red (PICCS)
Other names	1-(o-Nitro-p-tolylazo)-2-naphthol 1-[(2-Nitro-4-methylphenyl)azo]-2-naphthol 2-Nitro-p-toluidine-2-naphthol
Chemical group (DSL stream)	Discrete organics
Major chemical class or use	Azo compounds; naphthalenes
Major chemical sub-class	Beta-naphthol pigment
Chemical formula	C ₁₇ H ₁₃ N ₃ O ₃
Chemical structure	
SMILES	O=N(=O)c(c(N=Nc(c(c(ccc1)cc2)e1)c2O)ccc3C)c3
Molecular mass	307.31 g/mol

¹ DSL (Domestic Substances List).

² Source: National Chemical Inventories (NCI). 2006: AICS (Australian Inventory of Chemical Substances); ASIA-PAC (Asia-Pacific Substances Lists); ECL (Korean Existing Chemicals List); EINECS (European Inventory of Existing Commercial Chemical Substances); ENCS (Japanese Existing and New Chemical Substances); NZIoC (New Zealand Inventory of Chemicals); PICCS (Philippine Inventory of Chemicals and Chemical Substances); and TSCA (Toxic Substances Control Act Chemical Substance Inventory).

Physical and Chemical Properties

The pigment industry synthesizes organic pigments that have low to very low solubilities in nearly all solvents (i.e., $< 1 \text{ mg L}^{-1}$ to $< 0.01 \text{ mg L}^{-1}$). This arises from the desire of the industry to produce chemicals that will retain their colour for a long time and in any type of material. Low solubility is enhanced by designing chemicals that have strong interactive forces within and between molecules. For beta-naphthol compounds, this is achieved by the intramolecular, bifurcated hydrogen bonds. Although the structure of Pigment Red 3 is often depicted as in Table 1, based on the measured bond lengths, the keto-hydrazone tautomer was found to be favoured. The keto-hydrazone tautomer is different with respect to certain bonds. Namely, there is a ketone oxygen on the naphthalene ring instead of the hydroxyl group, a double bond exists between the nitrogen and the naphthalene ring and the azo bond is a single bond (Figure 1). This structure creates bifurcated hydrogen bonds between the ortho substituents on the phenyl ring (like $-\text{Cl}$, or $-\text{NO}_2$), the azo group and the ketone oxygen on the naphthalene group. The molecules may be linked by weak van der Waals forces and charge-transfer forces causing the molecules to stack in columns within a crystal (Herbst and Hunger 2004; Whitaker 1978; Lincke 2003).

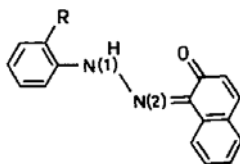


Figure 1. Beta-naphthol pigment structure (Whitaker 1978)

As is the case with the majority of organic pigments, beta-naphthol pigments generally do not exist as individual molecules but are principally particles in the submicron range. The pigment powder is typically composed of primary particles (i.e., the crystal lattice of a pigment), aggregates and agglomerates. Manufacturers usually provide the physical specifications of their pigments, which include the average particle size of the pigment powder (see Table 2a). In doing so, users can determine which pigment is the most appropriate to colour their product(s), since performance is chiefly controlled by the particle size distribution (Herbst and Hunger 2004).

Table 2a contains modelled and experimental physical and chemical properties of Pigment Red 3 that are relevant to its environmental fate. Modelled estimates for these properties are typically generated using quantitative structure-activity relationship (QSAR) models. These models, in turn, base their predictions on the characteristics of the individual molecules. Pigment Red 3 is expected to exist in the crystalline form in the environment: therefore there is uncertainty associated with the modelled physical and chemical data. The modelled $\log K_{OW}$ of 6.45 (KOWWIN 2000) used for categorization implies that the solubility of Pigment Red 3 is much higher in octanol than in water. Experimental solubility data, however, reveal that the difference in the solubility in the two solvents is not that sizeable, indicating that the modelled partition coefficient is likely overestimated. The ratio $\log (C_O/C_W)$ has been estimated from the experimental

solubilities of Pigment Red 3 in octanol (C_o) and water (C_w), and this experimentally derived ratio has been preferred over the model-derived $\log K_{OW}$ for this pigment. The modelled estimate of $\log K_{OW}$ has therefore been disregarded for this assessment, and the ratio $\log (C_o/C_w)$ has been presented instead (Table 2a).

The experimental solubilities in Table 2a have been determined using an aggressive approach with long contact times between pigment particles and the solvent, and a filtration step removing as much of the particulate matter in the suspension as possible. These studies have been critically reviewed, and although none reported using reference chemicals of known solubilities, they were determined to have a satisfactory degree of reliability for the present risk assessment (Appendix 1).

Table 2a. Physical and chemical properties for Pigment Red 3

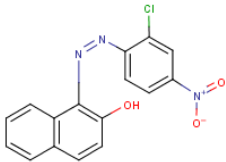
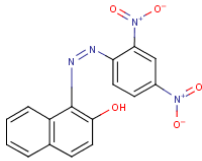
Property	Type	Value	Temperature (°C)	Reference
Physical state	Experimental	Red powder		MSDS 2004
Average particle size (nm)	Experimental	255–370		Clariant 2007
	Experimental	260–530		NPIRI 2000
Melting point (°C)	Experimental	276		Danish EPA 1998
	Modelled	200.3		MPBPWIN 2000
Boiling point (°C)	Experimental	NA		
	Modelled	473.67		MPBPWIN 2000
Density (kg/m ³)	Experimental	1400 (1.4 g/cm ³)		MSDS 2004
	Modelled	NA		
Vapour pressure (Pa)	Experimental	NA		
	Modelled	3.52×10^{-8} (2.64×10^{-10} mm Hg)	25	MPBPWIN 2000
Henry's Law constant (Pa·m ³ /mol)	Experimental	NA		
	Modelled (Bond estimation method)	6.59×10^{-8} (6.5×10^{-13} atm·m ³ /mol)	25	HENRYWIN 2000
	Modelled (Group estimation method)	1.19×10^{-7} (1.17×10^{-12} atm·m ³ /mol)	25	HENRYWIN 2000
Log K_{ow} (Octanol-water partition coefficient) (dimensionless)	Experimental	NA		
	Modelled	NA ¹		KOWWIN 2000
Log (C_o/C_w) (dimensionless)	Experimental	3.73		Calculated based on data in Table 2a
Log K_{oc} (Organic carbon-water partition coefficient – L/kg) (dimensionless)	Experimental	NA		
	Modelled	NA		
Water solubility	Experimental	0.0033	23–24	Study Submission

Property	Type	Value	Temperature (°C)	Reference
(mg/L)				2007a
	Modelled	0.49	25	WSKOWWIN 2000
Other solubilities (mg/L)	Experimental (octanol)	17.9	23–24	Study Submission 2007b
pK _a (Acid dissociation constant) (dimensionless)	Modelled	13.5		ACD 2005
	Experimental	NA		

NA: Not available; NA¹: Not applicable (see text above for details).

Octanol and water solubility studies were performed on two structurally similar substances, Pigment Red 4 and Pigment Orange 5 (CAS RN 2814-77-9 and CAS 3468-63-1 respectively). The results support the low water solubility and octanol solubility obtained for Pigment Red 3. The ratio log (C_o/C_w) has been calculated as a surrogate for log K_{OW} (Table 2b).

Table 2b. Empirical physical and chemical property data for analogues Pigment Red 4 and Pigment Orange 5

Chemical structure			
<p>Analog Pigment Red 4^a CAS RN 2814-77-9</p> 		<p>Analog Pigment Orange 5^b CAS RN 3468-63-1</p> 	
Property	Value	Temperature (°C)	Reference
Pigment Red 4			
Melting Point (°C)	276		NPIRI 2000
Water solubility (mg/L)	0.0033	22–23	Study Submission 2007c
Octanol solubility (mg/L)	9.4	22–23	Study Submission 2007d
Log (C _o /C _w) (dimensionless)	3.5		Calculated
Pigment Orange 5			
Melting Point (°C)	302		Danish EPA 1998
Water solubility (mg/L)	0.0068	26–27	Study Submission 2007e
Octanol solubility (mg/L)	1.76	26–27	Study Submission 2007f
Log (C _o /C _w) (dimensionless)	2.4		Calculated

^a Pigment Red 4 differs from Pigment Red 3 in two chemical features: a Cl is substituted for a NO₂ and a NO₂ is substituted for a methyl group on the terminal benzene ring.

^b Pigment Orange 5 differs from Pigment Red 3 in one chemical feature: a NO₂ is substituted for a methyl group on the terminal benzene ring.

Sources

Pigment Red 3 is not naturally produced in the environment.

Based on the results of an industry survey conducted for the 2006 calendar year under a notice issued pursuant to section 71 of CEPA 1999 (Canada 2007b), twelve companies were identified as either manufacturers or importers or users of Pigment Red 3 whether alone, in a product, in a mixture or in a manufactured item in a quantity ≥ 100 kg, or used in a quantity ≥ 1000 kg at any concentration (Environment Canada 2008a). Only one company reported manufacturing Pigment Red 3 in Canada above the 100 kg reporting threshold (100 000 – 1 000 000 kg). Of this manufactured quantity, all but between 30,000 and 50,000 kg was exported from Canada. Additionally, six companies reported importing above the 100 kg reporting threshold while one company reported importing below, with total reported imports ranging between 10 000 and 100 000 kg. Seven companies reported using Pigment Red 3 above the 1000 kg threshold, and two reported use below the threshold. The total amount in use for 2006 was approximately 40,000 kg.

Pigment Red 3 is one of the twenty highest-produced organic pigments (by volume) in the world (Herbst and Hunger 2004). The use of Pigment Red 3 has been reported in the United States under the Inventory Update Reporting program to be between 225 and 450 tonnes during the following years: 1990, 1994, 1998 and 2002. In the European Union, Pigment Red 3 is a Low Production Volume Chemical, indicating that production has been estimated to be in the order of 10 to 1000 tonnes per year. The database for Substances in Preparations in Nordic Countries indicates that in 2004, approximately 72.3 tonnes were used in Denmark, 18.4 tonnes were used in Norway and 6 tonnes were used in Sweden (SPIN 2006).

Uses

In Canada, Pigment Red 3 is used in a variety of industrial and commercial applications as either an organic pigment in primarily alkyd (and recently, now acrylic) enamel paints (gloss) for both interior and exterior applications including anti-rust proofing, as well as in plastics, printing inks (toner), textiles and polyurethane coatings (Environment Canada 2008a).

The only manufacturer of Pigment Red 3 in Canada uses 95% of the manufactured pigment for paint end-use products. Industrial applications include manufactured or imported industrial enamel paints for use on metal tools or equipment. Other industrial applications are in plastic compounding- plastic colouration and the manufacture of industrial printing inks. Commercial applications include sale as commercial enamel

paints for both interior and exterior use as decorative paints or acrylic-based coatings or sealants.

Exposures to Pigment Red 3 by the general population of Canada are limited to the situations where commercial products containing Pigment Red 3. According to the section 71 survey (CEPA 1999) and public comments received, types of paints containing Pigment Red 3 could be for brush, roller, and aerosol applications.

In Canada, three notifications of cosmetic products were reported to contain Pigment Red 3; one nail polish and two bar soaps, at concentrations less than 0.1 wt/wt % (CNS 2008). One of two soaps was notified as intended use for children. Pigment Red 3 is not listed on Health Canada's Cosmetic Ingredient Hotlist of prohibited or restricted ingredients for Canadian cosmetics.

Pigment Red 3 also has known uses as a colouring agent in cosmetics in some other countries. In Europe, Pigment Red 3 (CI 12120) is allowed exclusively in cosmetic products intended to come into contact only briefly with the skin as per Council Directive 76/768/EEC, Annex IV Part I (European Commission 1976). In contrast to Canada, Pigment Red 3 is not approved for cosmetics use in the United States (CTFA 2008).

Pigment Red 3 is listed on the Pest Management Regulatory Agency's list of formulants (List 2) which contains formulants that are considered potentially toxic (PMRA 2007, PMRA 2007a). It is reported to be used in two pest control products (antifouling paints) at concentrations of less than 1% of the total product (2008 personal communication with PMRA).

Pigment Red 3 has not been reported in food products (Environment Canada 2008a).

Releases to the Environment

Pigment Red 3 was identified to be primarily used in paints according to the results of the CEPA section 71 notice (Environment Canada 2008a). The importation, manufacture and use patterns of Pigment Red 3 reported for 2006 in Canada were used to estimate releases.

Mass flow tool

To estimate potential release of the substance to the environment at different stages of its life cycle, a mass flow tool was developed (Environment Canada 2008b). Empirical data concerning releases of specific substances to the environment are seldom available. Therefore, for each identified type of use of the substance, the proportion and quantity of release to the different environmental media are estimated, as are the proportion of the substance chemically transformed or sent for waste disposal. Unless specific information on the rate or potential for release of the substance from landfills and incinerators is

available, the mass flow tool does not quantitatively account for releases to the environment from disposal.

Assumptions and input parameters used in making these estimates are based on information obtained from a variety of sources including responses to regulatory surveys, Statistics Canada, manufacturers' websites and technical databases. Of particular relevance are emission factors, which are generally expressed as the fraction of a substance released to the environment, particularly during its manufacture, processing, and use associated with industrial processes. Sources of such information include emission scenario documents, often developed under the auspices of the Organisation for Economic Co-operation and Development (OECD), and default assumptions used by different international chemical regulatory agencies. It is noted that the level of uncertainty in the mass of substance and quantity released to the environment generally increase further down the life cycle.

Table 3. Estimated releases and losses of Pigment Red 3 to environmental media, transformation and distribution to management processes, based on the Mass Flow Tool

Fate	Proportion of the mass (%)¹	Major life cycle stage involved²
Releases to receiving media:		
To soil	1.6	Industrial use, consumer use
To air	0.0	-
To sewer ³	4.7	Production, formulation, industrial use, consumer use
Chemically transformed	0	-
Transferred to waste disposal sites (e.g., landfill, incineration)	93.7	Production, formulation, industrial use, consumer use, waste disposal

¹ For Pigment Red 3, information from the following OECD emission scenario documents was used to estimate releases to the environment and distribution of the substance as summarized in this table: Transport and Storage (Brooke and Crookes 2007), Coating Industry (OECD 2006), Adhesive Formulation (OECD 2007) Textile Finishing (OECD 2004). Values presented for release to environmental media do not account for possible mitigation measures that may be in place in some locations (e.g., partial removal by sewage treatment plants). Specific assumptions used in derivation of these estimates are summarized in Environment Canada 2007.

² Applicable stage(s): production-formulation-industrial use-consumer use-service life of article/product-waste disposal.

³ Wastewater before any form of treatment

Results indicate that Pigment Red 3 can be expected to be found largely in waste management sites (94%) due to waste produced during manufacturing of the pigment, manufacturing of paints, use of these paints and the eventual disposal of manufactured items containing them. A small fraction of solid waste is incinerated which is expected to result in transformation of the substance. Based largely on information contained in OECD emission scenario documents for processing and uses associated with this substance, it is estimated that 1.6% and 4.7% of Pigment Red 3 may be released to soil and wastewater, respectively. Releases of Pigment Red 3 to soil are expected to occur from flaking and chipping of paints during industrial and consumer use. Releases of Pigment Red 3 to water are predicted to be mostly due to releases during pigment manufacturing (such as transfer line cleaning) and also from transfer lines, cleaning

equipment and from transferring the substance from the vessel to bags during formulation (e.g., paint manufacturing). Releases to water are also predicted to occur from brush residues during industrial and consumer use.

Although no information is available on the quantity of importation of consumer products containing Pigment Red 3, it is anticipated that the quantities of releases to the various environmental media would not be significantly different from those estimated here. However, the quantities sent for waste management would be higher if importation of these products were taken into consideration.

Environmental Fate

The very low modelled vapour pressure of 3.52×10^{-8} Pa and a negligible Henry's Law constant of $\sim 10^{-8}$ Pa·m³/mol for Pigment Red 3 are consistent with the fact that it is a large and complex molecule (Baughman and Perenich 1988; Danish EPA 1998). This pigment is not expected to volatilize at environmentally realistic temperatures and will thus not be subject to long range atmospheric transportation.

The particulate character of Pigment Red 3 should have a key influence on its fate in the environment. Its particle size and density, together with its chemical stability and low aqueous solubility indicate that it will partition by gravity to sediments if released to surface waters, and will tend to remain in soils if released to terrestrial environments.

Persistence and Bioaccumulation Potential

Environmental Persistence

Because of its very low solubility in water, this pigment may be considered to be not available for aerobic biodegradation if released to water during pigment manufacturing. Jaffe (1996) has stated that once a pigment is incorporated into a matrix (e.g., paint), it is expected to be durable and withstand the combined chemical and physical stresses of weather, solar radiation, heat, water and industrial pollutants. Therefore, direct contact with biota probably does not occur when the pigment is incorporated in paint and it is not expected that the pigment would be susceptible to abiotic degradation.

Industries that manufacture pigments recognize that their substances are persistent. For example, the Color Pigments Manufacturers Association, Inc. (CPMA 2003) has indicated that pigments are designed to be durable or persistent in the environment in order to provide color to finished coatings, inks and paints.

The environmental persistence of beta-naphthol pigments, such as Pigment Red 3, in anoxic environments is an important area of uncertainty. Azo dyes are reported to be degraded in anoxic waters and sediment via anaerobic reduction of the azo bond (-N=N-) (Weber and Wolfe 1987). A mutagenic potential is attributed to their breakdown products which include aromatic amines (Van der Zee 2002). Beta-naphthol pigments have azo chromophores in their structure as well; however, no documentation has been found regarding a possible degradation potential of these pigments in the absence of oxygen. In principle, the crystal would have to dissolve first, releasing its constituent molecules. Then, the azo bonds in these molecules would be available for reduction. However given its limited solubility, it is expected that only a very small proportion of the pigment would be reduced in this manner.

Some disperse azo dyes have been shown to undergo anaerobic degradation in sediment at depth where anoxic conditions persist (Yen et al 1991, Baughman and Weber 1994, Weber and Adams 1995). Disperse dyes and pigments are expected to eventually settle to the aerobic layers of surface sediment where they will persist until sediment burial creates conditions suitable for reducing conditions. The rate of sediment deposition varies from site to site and thus is very difficult to ascertain the residence time of dyes in anaerobic sediment layers as a function of sediment burial. It is likely however, that this is much greater than 365 days. Once under anaerobic or reducing conditions, azo dyes may undergo degradation to substituted aromatic constituents. At depth, these biodegradation transformation products are not expected to present a high degree of exposure potential to most aquatic organisms and therefore not likely to present an ecological concern. It is also expected that if the azo pigment is reduced, it would not likely present an ecological harm.

Based on the weight of evidence provided from the above-described literature, Pigment Red 3 is considered to meet the persistence criteria defined in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential for Bioaccumulation

There is a predictable relationship between K_{OW} and the bioconcentration factor in lipids (Mackay 1982). The ratio $\log(C_O/C_W)$ has been estimated from the experimental solubilities of Pigment Red 3 in octanol (C_O) and water (C_W) (Table 2a), and this experimentally derived ratio is preferred over the model-derived $\log K_{OW}$ for this pigment. This approach is supported by the observation that partitioning into octanol is a good indicator of a substance's potential to partition into the lipid phase of aquatic biota (Bertelsen et al. 1998) and, for pigments, the observation that a reduced solubility in octanol translates into a similarly reduced bioconcentration factor (BCF) and bioaccumulation factor (BAF) in an aquatic organism (Banerjee and Baughman 1991).

A revised set of BCF and BAF estimates for Pigment Red 3, different from those used during categorization, have been obtained from quantitative structure-activity relationship (QSAR)-based bioaccumulation models, using the experimentally based value \log

(C_o/C_w) in place of the QSAR-estimated $\log K_{ow}$. Table 4 shows that the revised BCF and BAF estimates are mostly below 1000 for Pigment Red 3, except for one prediction from the OASIS model, which is slightly over 1000. Similar $\log (C_o/C_w)$ values have been derived from experimental solubilities for reasonably close analogues, Pigment Red 4 and Pigment Orange 5 (Table 2b), supporting the low bioaccumulation potential of Pigment Red 3.

Table 4. Modelled bioaccumulation data for Pigment Red 3

Test organism	Endpoint	Value wet weight (L/kg)	Reference
Fish	BAF	404	Gobas BAF T2MTL (Arnot and Gobas 2003)
Fish	BCF	299	Gobas BCF T2LTL (Arnot and Gobas 2003)
Fish	BCF	1084	OASIS Forecast 2005
Fish	BCF	10*	BCFWIN 2000

*Default value for non-ionizable azo pigments.

Pigment Red 3 is therefore expected to present a low bioaccumulation potential, because of its limited affinity for the lipid phase of living organisms. This is in agreement with the conclusion of a Danish assessment report (Danish EPA 1998) that organic pigments are generally not bioaccumulative.

The results of QSAR models indicate that Pigment Red 3 does not meet the bioaccumulation criterion (BCF, BAF ≥ 5000) as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential to Cause Ecological Harm

Ecological Effects Assessment

A - In the Aquatic Compartment

There is experimental evidence that Pigment Red 3 does not cause acute harm to aquatic organisms at the level of saturation. Furthermore, predicted ecotoxicity values were obtained using the experimental $\log (C_o/C_w)$ of Pigment Red 3.

The effect of a saturated solution of Pigment Red 3 on the immobilisation of *Daphnia magna* was determined under static conditions over 48 hours (Table 5a). Twenty test organisms were exposed to the saturated solution and a control. Water quality parameters were measured at the start and end of the test. The pH was maintained between 7.80 and 7.88 and oxygen between 8.49 and 8.61 mg/L. The temperature ranged from 18 to 22°C. Saturation was achieved by shaking the stock solution for 24 hours and removing undissolved particles by centrifugation. The concentration of the pigment in solution was measured by dissolved organic carbon (DOC) analysis at the start and end of the test. It

was observed that 0.6 mg/L DOC were present at the beginning and end of the test, indicating that the concentration of the pigment was maintained throughout the test. Based on the measured DOC, the concentration of pigment at saturation is estimated to be approximately 0.9mg/L. No biologically significant effects (immobilization) were observed at saturation. This study is considered to be of high reliability for the present assessment as good laboratory practices (GLP) were followed, control and reference toxicants were used and the dissolved organic carbon concentration was measured at the beginning and end of the experiment (Appendix 1G). However, according to the guidance provided by the OECD for sparingly soluble substances, when a substance is found to have no effects at saturation, this saturation concentration is typically below the water solubility value obtained in a water solubility test (OECD 2000). The water solubility of Pigment Red 3 was measured to be 3.3µg/L. Therefore the measured DOC in this test may not be representative of the dissolved concentration only, but a measure of the pigment particles and perhaps a small fraction of dissolved pigment. It is not expected that the maximum solubility would have been achieved by shaking the stock solution for 24 hours as the pigment was shaken in water for 2 hours at 30°C and then for 70 hours at 23–24°C in the solubility test, which still resulted in a residue of undissolved colorant on the 0.05µm filter. Therefore, it is expected that undissolved pigment was also present in the toxicity test. This assumption that the DOC was not representative of only the dissolved concentration is further supported by the results of a toxicity test for Pigment Orange 5.

In a similar toxicity test on the analogue, Pigment Orange 5 (CAS RN 3468-63-1), it was also found that no biologically significant effects were observed in *Daphnia magna* at saturation (Table 5a). This study was also considered to be of high reliability (Appendix 1H). Rather than using centrifugation to separate the undissolved fraction, a 0.45µm membrane filter was used. The average particle size for Pigment Orange 5 is 0.285µm; therefore, the filter would not have removed most particles. The DOC was also measured in this test and was found to correspond to a concentration of 1.6mg/L of pigment. Since the filter was too large to capture the pigment particles, it is expected that 1.6mg/L is the concentration of the dissolved pigment and the particles. As the concentrations found for Pigment Red 3 and Pigment Orange 5 are similar in the toxicity tests, and both exceed their water solubilities, this study supports the conclusion that the results of the Pigment Red 3 test reflect the toxicity of both the pigment particles and a small fraction of dissolved pigment. Therefore, the dissolved and particulate forms of Pigment Red 3 are expected to have a negligible to low acute toxicity to *Daphnia magna*.

Aquatic toxicity predictions, recalculated using $\log(C_o/C_w)$, were obtained from the ECOSAR program (ECOSAR 2004). It is assumed that Pigment Red 3 has a narcotic mode of action (MOA) similar to that of phenols. However, the ASTER (1999) model predicted the MOA as “uncoupling of oxidative phosphorylation” for this pigment, in addition to narcosis. An application factor of 100 was therefore applied to the ECOSAR estimate to extrapolate from baseline toxicity to this more toxic MOA. It should be noted that these MOAs are predicted for the solubilized molecule which is likely released in very low amounts in solution as suggested by the solubility test in water. Furthermore, the training sets for ECOSAR phenols or ASTER do not contain pigments, introducing

more uncertainty in these estimates. Table 5b presents these modelled ecotoxicity results, which are consistent with the empirical studies indicating that there would be no acute effects at saturation.

Table 5a. Experimental aquatic toxicity value for Pigment Red 3 and analogue Pigment Orange 5

Substance	Organism	Test type	Endpoint	Duration	Value	Reference
Pigment Red 3	Daphnid	Acute	EC ₅₀ ¹	48 hours	No effect at saturation (0.9mg/L)	Study Submission 2007g
Pigment Orange 5	Daphnid	Acute	EC ₅₀ ¹	48 hours	No effect at saturation (1.6mg/L)	Study Submission 2007h

¹ Immobilization

Table 5b. Modelled aquatic toxicity values for Pigment Red 3

Organism	Endpoint	Duration	Value (mg/L)	Chemical class/mode of action	Reference
Fish	LC ₅₀	14 days	13.642 ¹	Neutral Organic SAR (baseline toxicity)	ECOSAR 2004
Fish	LC ₅₀	14 days	0.1364	Uncoupling of oxidative phosphorylation	Calculated ²
Fish	LC ₅₀	96 hours	4.049 ¹	Phenols	ECOSAR 2004
Daphnid	LC ₅₀	48 hours	3.336 ¹	Phenols	ECOSAR 2004
Green algae	EC ₅₀	96 hours	4.776 ¹	Phenols	ECOSAR 2004

¹ Chemical may not be soluble enough to measure this predicted effect

² Calculated by applying an extrapolation factor of 100 to the baseline toxicity prediction from ECOSAR 2004 to account for a mode of action other than narcosis alone (uncoupling of oxidative phosphorylation) as predicted by ASTER 1999.

Chronic exposure to Pigment Red 3 is likely to be low in water due to its low water solubility, relatively low potential for bioaccumulation, high molecular weight and particulate nature.

Overall, experimental and modelled toxicity data indicate that Pigment Red 3 is predicted to have a negligible to low potential for toxicity to aquatic organisms (LC₅₀/EC₅₀ above saturation).

B - In Other Environmental Compartments

No empirical or predicted effects data for non-aquatic organisms were identified for this compound. Pigment Red 3 is expected to reside in sediment or soil: however, effect levels in these media have not been identified.

Ecological Exposure Assessment

No data have been found regarding concentrations of Pigment Red 3 in the Canadian environment. The mass flow tool estimated that more than 90 percent of the mass of this pigment ends up in waste disposal facilities. Off-site chemical migration from these facilities is unlikely, or can be predicted to be minor, because of the negligible geochemical mobility of the pigment indicated by its very low solubility in water and in organic solvents. Consequently, it is anticipated that there are negligible releases associated with the waste management stage of this substance.

The mass flow tool estimated that up to about 5 percent of the total mass of Pigment Red 3 in use could be released to water throughout the life cycle of the substance. Available industrial information suggested that these releases would be mostly generated during pigment manufacturing and during industrial processes using the pigment to manufacture other coloured products, such as paint (Environment Canada 2007). A conservative, site-specific scenario was developed to obtain a predicted environmental concentration (PEC) at the discharge site of the pigment manufacturer (Environment Canada 2008c). The site of the manufacturer was selected, because this company handles the highest quantity of Pigment Red 3 in Canada, as reported in response to the industrial survey for 2006 (Environment Canada 2008a). In addition, the scenario conservatively assumed that releases from transport and handling, paint manufacturing and transfer line clean-out from activities of the importers of the pigment were also discharged to the treatment plant used by the manufacturer. The removal rate of Pigment Red 3 from this treatment plant is estimated to be 78% (ASTreat 2006). Using these assumptions, it was predicted that the PEC resulting from these combined releases (assuming instantaneous dilution) would be less than 1mg/L. Due to the confidential nature of the manufactured and imported quantities, the actual PEC cannot be disclosed.

Pigment Red 3 is used in paints and coatings. Therefore it is possible that exposure may occur from its use in these products. No data concerning concentrations of this substance in water in Canada have been identified. Environmental concentrations are, therefore, estimated from available information, including estimated substance quantities, release rates, and receiving water bodies. Environment Canada's tool to estimate down-the-drain releases from consumer uses (Mega Flush) was employed to estimate the potential substance concentration in multiple water bodies receiving sewage treatment plant effluents to which consumer products containing the substance may have been released (Environment Canada 2008d). The tool is designed to provide these estimates based on conservative assumptions regarding the amount of chemical used and released by consumers. It was assumed that primary and secondary sewage treatment plant (STP) removal rates are 55.3% and 78.1% respectively (ASTreat 2006) and that losses from use are 1.5%, consumer use of the substance is 365 days/yr, and the flow rate used at all sites is in the 10th percentile. These estimates are made for approximately 1000 release sites across Canada, which account for all of the major STPs in Canada.

The equation and inputs used to calculate the predicted environmental concentration (PEC) of Pigment Red 3 in the receiving water bodies are described in Environment

Canada (2008d). A scenario was run assuming a total consumer use quantity of 100 000 kg, derived from the upper limit of the quantity range of the total manufacture and import, less the export. Using this scenario, the tool estimates that the PEC in the receiving water bodies ranges from 0 to 0.00095 mg/L.

Characterization of Ecological Risk

The approach taken in this ecological screening assessment was to examine the available scientific information and develop conclusions based on a weight-of-evidence approach and using precaution as required under CEPA 1999. Particular consideration has been given to risk quotient analysis, persistence, bioaccumulation, inherent toxicity, sources and fate in the environment.

Pigment Red 3 is determined to be persistent, based on published evidence and comments submitted by industry. However, based on observations of its very low solubility in octanol and low modelled BCFs, it has been determined not to be bioaccumulative in accordance with the *Persistence and Bioaccumulation Regulations* of CEPA 1999 (Canada 2000).

An experimental toxicity study performed on *Daphnia magna*, revealed that no effects were observed at 0.9mg/L. A slightly more potent toxicity was predicted using aquatic toxicity models, resulting in a LC₅₀ of 0.136 mg/L. The modelled result is felt to address an endpoint that is not captured by existing experimental results. As noted earlier, the predicted effect of Pigment 3 as a neutral organic causing narcosis (LC₅₀ of 13.6 mg/L) was further extrapolated using an application factor of 100 to estimate the level at which the substance is likely to induce uncoupling of oxidative phosphorylation. This results in a critical toxicity value (CTV) of 0.136 mg/L. A further application factor of 100 is used to account for uncertainties in extrapolation from acute to chronic effects, lab to field effects and for using data for the analogue substance. The resulting predicted no effects concentration (PNEC) is 0.0014 mg/L and is deemed very conservative due to the multiple application factors applied to the original prediction.

When applying this PNEC to the site-specific scenario developed for industrial releases, and the results of the Mega Flush tool, the risk posed by exposure to Pigment Red 3 is expected to be low. In the first case, the site specific exposure scenario generated a risk quotient of less than 1 at the discharge point of the pigment manufacturer. In the second case, for exposure resulting from down-the-drain releases through consumer uses (very conservative scenario), the MegaFlush results estimate that the PNEC will not be exceeded at any sites (i.e., all risk quotients < 1). This indicates that down-the-drain consumer releases of Pigment Red 3 are not expected to cause harm to aquatic organisms.

Considering these findings, it is concluded that Pigment Red 3 is unlikely to be causing ecological harm in Canada.

Uncertainties in Evaluation of Ecological Risk

This section summarizes the key uncertainties associated with the risk assessment of Pigment Red 3.

Pigment Red 3 is expected to partition primarily to sediment; however, data on its fate and toxicity in sediments are lacking. Specifically, the long-term stability of Pigment Red 3 in anoxic sediments, as well as in anoxic layers in the soil column of waste disposal sites, is largely unknown. It has been assumed that the crystalline structure of Pigment Red 3 would be maintained in these compartments, thereby remaining unavailable to sediment-dwelling organisms and unavailable for reduction of the azo bond, which could release bioavailable aromatic amines. Although acute and chronic toxicity data are not available for sediment or soil-dwelling organisms, toxicity is expected to be low based on information for aquatic organisms.

Organic pigments, such as Pigment Red 3, may have a certain proportion of their particle size spectra in the nanoparticle range (e.g., Table 2a). Nanoscale materials are informally defined as substances having at least one dimension of less than 100 nm. Evidence is accumulating to the effect that nanoparticles can be absorbed by non-specific biouptake pathways such as pinocytosis (Leroueil et al. 2007). Presently, the bioaccumulation mechanisms and potential of these particles are poorly understood, as is the nature of the relationship between their bioaccumulation and their toxicity. Furthermore, certain less commonly considered environmental fate processes may have an important influence on the propensity of the pigment nanoparticles to be taken up by biota (e.g., importance of aggregation in nature: Wiesner et al. (2006)).

Potential to Cause Harm to Human Health

Exposure Assessment

Derivation of upper-bounding estimates of daily intake of Pigment Red 3 from environmental media by the general population of Canada was precluded in this assessment as no information of Pigment Red 3 in environmental media was available. Consequently, only exposure estimates from use of known consumer products are presented in this section.

Exposure scenarios were developed for three types of paints (high solvent, high solid, and aerosol) as well as for two cosmetic products (nail polish and soap) using ConsExpo 4.1 consumer product exposure modelling (RIVM 2006a). Details of the scenarios are presented in Appendix 2. For use of soap, scenarios were developed for a “solid soap” for washing hands and showering by adults and children. Inhalation and dermal routes of exposure for all paint scenarios were considered while only dermal exposure scenario was developed for use of soap.

In the absence of dermal absorption rate data for Pigment Red 3, Sudan I (1-Phenylazo-2-naphthol, CI 12055), was used as its analogue to estimate uptake rate. According to the available study data, $26.4 \pm 6.7\%$ of the applied radioactive isotope labelled Sudan I applied to human skin was absorbed in 24 hours in an *in vitro* percutaneous absorption study (Collier et al. 1993). Therefore, dermal absorption rate (uptake rate) of $26.4 \pm 6.7\%$ was used to estimate dermal exposure to Pigment Red 3.

According to the information submitted under the section 71 of CEPA 1999, typical concentrations of Pigment Red 3 in paint products range from 1 to 15 wt/wt% (Environment Canada 2008a). Consequently, for conservative estimation of exposure during use of paint product, the weight fraction of 15 wt/wt% is used. In the case of aerosol paints where its use in Canada has been confirmed, the maximum concentration of 10% was used as a conservative estimate (Appendix 2).

Exposure estimates during use of solvent-rich and high-solid paints via inhalation route were in the same order of magnitudes (1.4×10^{-8} and 2.2×10^{-8} mg/kg-bw per event, respectively), whereas use of aerosol paints resulted in a much higher exposure estimate of 0.1 mg/kg-bw per event. In contrast exposures via dermal route were estimated to be approximately 2.0 mg/kg-bw per event for all three types of paint, a significantly higher value than estimated for inhalation exposures.

Based on information received under the section 71 survey, once the pigment is dispersed in the paint formulation (in either water-based or solvent/alkyd-based paints), exposure potential to the pigment is lowered because the pigment particles become encapsulated in the paint resin (Environment Canada 2008a). The low exposure estimates obtained by ConsExpo 4.1 modelling agree with this statement for solvent-rich and high-solid paints. In the case of aerosol paints, exposure to Pigment Red 3 by inhalation is estimated to be much higher and most likely due to inhalation of resin droplets which itself contain Pigment Red 3 in addition to any exposure from vapours.

Exposure during use of nail polish resulted in an inhalation estimate of 2.4×10^{-12} mg/kg-bw per event and a dermal estimate of 1.8×10^{-4} mg/kg-bw per event. The maximum dermal exposure during use of soap resulted in an estimated exposure of 4.4×10^{-3} mg/kg-bw per day for washing hands by adults. Children's use resulted in lower values. Exposure to Pigment Red 3 from the cosmetic products was estimated to be much lower than that from paint products. Based on the low number of notification of cosmetic products containing Pigment Red 3 in Canada (CNS 2008) and the low estimates of exposure, these products do not contribute significantly to consumer exposure.

Health Effects Assessment

Appendix 3 contains a summary of the available health effects information for Pigment Red 3.

The U.S. National Toxicology Program (NTP) had conducted two-year toxicology and carcinogenesis studies of Pigment Red 3 in F344/N rats and B6C3F1 mice. From these

two-year oral studies, NTP concluded that there was some evidence of carcinogenicity of Pigment Red 3 in male and female F344/N rats and B6C3F1 male mice, and no evidence of carcinogenic activity in female mice (NTP 1992). The International Agency for Research on Cancer (IARC) evaluated the carcinogenicity of Pigment Red 3 and concluded that Pigment Red 3 *cannot be classified as to its carcinogenicity to humans* (Group 3) based on the limited evidence in experimental animals and inadequate evidence in humans for the carcinogenicity (IARC 1993).

Pigment Red 3 has shown increased incidence of adrenal pheochromocytomas in male rats and of hepatocellular adenoma in female rats. Groups of 50 male and female Fisher 344 rats were fed diets containing 0 ppm, 6000 ppm, 12 500 ppm, 25 000 ppm (equivalent to 0, 325–378, 678–795, 1415–1598 mg/kg-bw/day, respectively) Pigment 3 for 2 years. In male rats, the incidence of benign adrenal pheochromocytomas was significantly increased in the mid- and high-dose groups compared to the control group (22/50, 29/50, 35/50, 34/50 for 0, 6000, 12 500, 25 000 ppm, respectively; $p=0.004$, logistic regression trend test). In female rats, hepatocellular adenoma occurred in a positive trend with significant increased incidence in the high dose (0/50, 0/50, 1/50, 10/50 for 0, 6000, 12500, 25000 ppm, respectively; $p=0.001$, logistic regression trend test). Other neoplastic effects included marginally significant increases of incidence of squamous cell papilloma or carcinoma in skin at a high dose (6/50 vs 1/50 in control group; $p=0.051$); and marginal increase incidence of carcinoma in the Zymbal's gland (3/50 vs 0/50 in control group) in male rats (NTP 1992).

In the complementary mouse two-year feed study, groups of 50 male and female B6C3F₁ mice were fed with diets containing 0 ppm, 12 500 ppm, 25 000 ppm, 50 000 ppm (equivalent to 0, 2482–2714, 4710–5491, 9933–12461 mg/kg-bw/day, respectively) Pigment Red 3. In male mice, significant increases of renal tubule adenoma were observed in the kidney in the high-dose group (0/50, 0/50, 0/50, 6/50 for 0, 12 500, 25 000, 50 000 ppm group, respectively; $p=0.017$, logistic regression trend test); and of follicular-cell adenoma in thyroid gland in the high-dose group (0/50, 0/49, 1/50, 5/50 for 0, 12 500, 25 000, 50 000 ppm, respectively; $p=0.027$, logistic regression trend test). No treatment-related tumours were seen in female mice (NTP 1992).

The genotoxicity results for Pigment 3 were equivocal in *in vivo* animal studies and negative in mammalian cell studies. Significant increase of DNA damage measured by comet assay was observed in colon tissue in male ddY mice 24 hours after oral treatment with 2000 mg/kg-bw Pigment Red 3. However, no DNA damage was observed in other organs examined (Tsuda et al. 2000), including those in which tumours have been observed in the two-year bioassay (NTP 1992). In another study, no significantly increased inductions of micronuclei in bone marrow cells and spleen were observed in mice after injection of Pigment Red 3 into the peritoneum (Barański et al. 1992, reviewed in Møller and Wallin 2000, dose not mentioned). In mammalian cell studies, Pigment Red 3 did not induce sister chromatid exchanges (SCE) or chromosomal aberrations (CA) in cultured Chinese hamster ovary cells in either the presence or absence of S9 (NTP 1992).

The mutagenicity of Pigment Red 3 in *Salmonella* bioassay was equivocal. Pigment Red 3 was mutagenic in *Salmonella typhimurium* strains TA100 and TA98 in the presence of induced hamster liver S9 fraction. A clear dose-response relationship was observed, although precipitate was formed at the highest test concentration. Equivocal results were seen in the presence of induced rat liver S9. No increases in gene mutation were observed in strains TA98 and TA100 without S9; or TA1535 and TA1537 with or without S9 (Mortelmans et al. 1986; NTP 1992). In other studies, negative *Salmonella* mutagenicity was also seen in TA98 and TA100 in the presence of metabolic activation (Miyagoshi et al. 1983; Barański et al. 1992, reviewed in Møller and Wallin 2000). Although IARC stated that these results indicated that “C.I. Pigment Red 3 was not mutagenic to *Salmonella typhimurium*, except in the presence of an exogenous metabolic system from hamster (but not rat) liver, when it was weakly mutagenic at precipitating doses”, Mortelmans et al. (1986) concluded that the mutagenicity results were positive. This was based on the criteria for mutagenicity evaluation for positive response “a dose-related, reproducible increase in the number of revertants over background, even if the increase was less than twofold.”

Although the mode(s) of action for induction of tumours has not been fully elucidated, and such analyses are beyond the scope of this screening assessment, based on the alerting structure for DNA reactivity in Pigment Red 3, aromatic nitro group and diazo bond, and the observed positive mutagenicity in *Salmonella* in the presence of hamster S9, the NTP concluded that Pigment Red 3 may be considered a genotoxic carcinogen (NTP 1992).

With respect to non-cancer critical effects, significantly increased incidences of non-neoplastic lesions were observed in the liver in male and female rats in the two-year NTP study (0 ppm, 6,000 ppm, 12 500 ppm, 25 000 ppm in diet, equivalent to 0, 325–378, 678–795, 1415–1598 mg/kg bw/day, respectively). At the lowest treatment dose of 6000 ppm (equivalent to 325–378 mg/kg bw/day), significantly increased incidence of liver lesions included eosinophilic focus (37/50 vs 6/50 in the control group, $p < 0.01$), mixed cell focus (24/50 vs 2/50 in the control group, $p < 0.01$), cystic degeneration (36/50 vs 9/50 in the control group, $p < 0.01$), and multifocal angiectasis (gross dilatation and often lengthening of a blood or lymph vessel, 20/50 vs 3/50 in the control group, $p < 0.01$) in male rats. The nonneoplastic effects in female rats include eosinophilic foci, mixed cell foci, granuloma and cholesterol pigmentation. Moreover, the significant increased incidences occurred at higher doses. Other nonneoplastic effects at the lowest dose included a dose-related increase in severity of nephropathy in both male and female rats; and increased incidence of hyperplasia in kidney papilla transitional epithelium in male rats (NTP 1992). In the mouse two-year feed study (0 ppm, 12 500 ppm, 25 000 ppm, 50 000 ppm in diet, equivalent to 0, 2482-2714, 4710-5491, 9933-12461 mg/kg bw/day, respectively), increased incidence of cytomegaly of the renal tubule epithelium (0/50, 40/50, 47/50, 46/50, respectively) was seen in male mice. In addition, a dose-related increase in severity of nephropathy and increased incidence of thyroid follicular cell hyperplasia were observed in both male and female mice (NTP 1992).

Thus, based on the liver lesions in the two-year study, the lowest-observed-(adverse)-effect level (LOAEL) was determined to be 325 mg/kg-bw/day (6000 ppm in diet, the lowest-dose tested).

In the subchronic study, Fisher 344 rats were exposed to Pigment Red 3 in diet (0 ppm, 3000 ppm, 6000 ppm, 12 500 ppm, 25 000 ppm, 50 000 ppm; equivalent to 0, 183–223, 408–478, 758–929, 1474–1704, 3183–3736 mg/kg-bw/day, respectively) for 90 days. The chemical-related histopathological lesions observed in both male and female rats, included hyperplasia in bone marrow; congestion and hematopoietic cell proliferation and pigmentation in the spleen; hematopoietic cell proliferation and pigmentation in the liver; and pigmentation in the kidney (Morgan et al. 1989; NTP 1992). At the lowest treatment dose of 3000 ppm in diet (183-223 mg/kg-bw/day), high incidence (10/10 animals vs 0/10 in the control group) of minimal hyperplasia in bone marrow and congestion, hematopoietic cell proliferation and pigment in the spleen were observed; other effects at this dose include significantly increased liver to body weight ratio (15%, $p < 0.05$, $n = 10$); a significantly increased number of reticulocyte ($p < 0.05$, $n = 10$) and a significant decrease of hematocrit ($p < 0.05$, $n = 10$). The dose-response relationships were observed for the increased liver weight ratio and the severity of lesions in the bone marrow and spleen. Thus, the oral LOAEL was determined to be 183 mg/kg-bw/day (3000 ppm in diet, the lowest-dose tested) based on observed hyperplasia in the bone marrow, histopathological lesions in the spleen and a significant increase in liver to body weight ratio in male rats.

In another subchronic study, B6C3F1 mice were fed with Pigment Red 3 in diet (0 ppm, 3000 ppm, 6000 ppm, 12 500 ppm, 25 000 ppm, 50 000 ppm; equivalent to 0, 502–666, 1048–1540, 3291–3121, 6298–6306, 10177–12520 mg/kg-bw/day, respectively) for 90 days. Chemical-related histopathological lesions were also seen in male and female B6C3F1 mice. The histopathological effects include a significant increase of hematopoietic cell proliferation in spleen and hematopoietic cell proliferation in the liver in male and female mice. Renal tubule epithelial cytomegaly was observed in male mice. Severe lesions were observed at higher concentrations (Morgan et al. 1989; NTP 1992). The oral LOAEL was determined to be 502–666 mg/kg-bw/day (3000 ppm in diet, the lowest-dose tested) in B6C3F1 mice for Pigment Red 3 based on the significant increase of hematopoietic cell proliferation in spleen.

In addition, significant increases of the liver to body weight ratio were seen in male Osborne-Mendel rats fed with a diet containing 0 ppm, 2500 ppm Pigment Red 3 for 90 days; no effects were observed on mortality, growth or haematology (Graham and Davis 1968). Compared with the NTP study, only one treatment dose was used in this study. Thus it is considered to be supplementary and no critical effect level is derived.

In a short-term repeat oral dose study, Fisher 344 rats and B6C3F1 mice were exposed to Pigment Red 3 in diet (0 ppm, 6000 ppm, 12 500 ppm, 25 000 ppm, 50 000 ppm, 100 000 ppm) for 14 days. The major toxic effects include dose-related decreases in erythrocyte counts, hematocrit concentrations and an increase in reticulocyte counts in rats. The oral LOAEL was identified as 738 mg/kg-bw/day (6000 ppm in diet, the lowest-dose tested) in females based on hematological effects (decrease in haemoglobin

concentration) (Morgan et al. 1989). Significantly decreased concentrations of hematocrit, hemoglobin and erythrocyte were observed in B6C3F1 male mice, but not with clear dose-related trends. The oral LOAEL for mice was about 1048 mg/kg-bw/day (6000 ppm in diet, the lowest dose tested) based on haematological effects (Morgan et al. 1989).

There was limited information about the bioavailability and metabolism of Pigment Red 3. Only a small proportion of Pigment Red 3 was detected in the urine and various tissues (blood, liver, kidneys or lungs) within 48 hours in an absorption study with male Fisher 344 rats by single gavage dose of 11.8 mg/kg Pigment Red 3 in corn oil, indicating a low absorption through the gastro-intestinal tract (El Dareer et al. 1984, reviewed in IARC 1993 and BIBRA 1996). About 72.4% of treated dose was recovered from the feces after 48 hours suggesting that the compound may be partially degraded by the intestinal bacteria (El Dareer et al. 1984). It is suggested that this azo dye may be reduced by bacterial flora in the gut and to some extent by enzyme in the liver to produce 4-methyl-2-nitro-aniline and 2-hydro- α -naphthylamine (Morgan et al. 1989). In this absorption study, only parent compound but not relevant metabolites were measured and labelled tracer was not used for this compound. In another *in vitro* percutaneous absorption study of Pigment Red 3 analogue, Sudan I, a skin permeability of 26.4 \pm 6.7% was determined for human skin exposed for 24 hours to radiolabelled Sudan I (Collier et al. 1993).

Pigment Red 3 causes slight irritation in the skin of humans. Slight erythema or erythema with edema were observed in patients with pigmented cosmetic dermatitis in patch or photopatch tests with Pigment Red 3 after application for 48 or 72 hours. Positive reactivity for skin irritation was 34.2% (13/38) (Sugai et al. 1977). Reddening and swelling of skin were seen in 5 of 155 patients with cosmetic dermatitis in a 48-hour patch test with 5% Pigment Red 3 in polyethylene glycol; slight reddening was seen in another 25 patients (MJCDRG 1978, cited in BIBRA 1996). Patch tests were carried out for several azo dyes in 8 patients suffering from pigmented contact dermatitis caused by Brilliant Lake Red R (CAS number 27757-79-5). Negative reaction was observed in the patch test with 1% Pigment Red 3 in petrolatum, whereas positive reactions were obtained in 1% Brilliant Lake Red R or 0.1% Sudan I in petrolatum. In addition, no skin reactions were seen in 28 healthy young female volunteers given a 48-hour patch test with 1% Pigment Red 3 in petrolatum (Kgzuka et al. 1980). It is suggested that Pigment Red 3 is a weak dermal irritant with a threshold concentration of > 1% in solvent.

The confidence in the toxicity database for Pigment Red 3 is considered to be moderate, as short-term, subchronic and chronic studies are available, but only for the oral route of exposure.

Characterization of Risk to Human Health

Based on consideration of relevant available information, including a weight-of-evidence based assessment by an international agency (IARC 1993), a critical effect for characterization of risk to human health for Pigment Red 3 is carcinogenicity. In a

two-year bioassay, exposure-related tumours were observed at multiple sites in both male and female rats, and in male mice (NTP 1992). The neoplastic effects included liver tumours in female rats, adrenal gland, skin and malignant Zymbal gland tumours in male rats, and tumours in the kidney and thyroid gland in male mice. No exposure-related tumours were observed in female mice.

Although a mode-of-action analysis for tumour induction at the multiple sites in rats and mice is considered to be beyond the scope of this Challenge screening assessment, in light of the equivocal evidence for genotoxicity based on a limited database (with positive results observed at high doses for mutagenicity in some strains of *Salmonella* under certain metabolic activation conditions and the induction of DNA damage in the colon of orally exposed mice), a mode of induction of tumours involving direct interaction with genetic material cannot be precluded.

With respect to consideration of critical non-cancer effects in a screening context, a margin-of-exposure (MOE) approach is applied for the risk characterization. Due to its uses and physical-chemical properties of low vapour pressure, Henry's law constant and solubility, exposure in the general environment is expected to be negligible. The major source of population exposure to Pigment Red 3 is from coming into dermal contact with consumer products. Due to the absence of dermal exposure toxicity studies, the critical effect levels from oral studies are applied to the MOE calculation and it is assumed that Pigment Red 3 induces similar toxicity through oral exposure or dermal exposure. In the available short-term, subchronic and chronic experimental studies the toxicological effects identified were observed at the lowest doses tested, with the lowest LOAEL of 183 mg/kg/day for histopathological and haematological effects in rats. Comparison of this critical effect level of 183 mg/kg/day and the upper-bounding estimate of dermal exposure of Pigment Red 3 from hand washing with a soap bar by adults (4.4×10^{-3} mg/kg-bw/day) results in a margin of exposure of approximate 41 600. When dermal exposure to Pigment Red 3 during use of paints (2.0 mg/kg-bw per event, 132 min exposure) is compared to the LOAEL of 738 mg/kg/day from a short term (14 days) oral study, a margin of exposure of approximately 370 is derived, however considering the conservatism of the exposure assessment, the actual exposures may be lower and would result in a higher MOE against this endpoint. It can be argued that in a highly refined Risk Assessment, a margin of exposure of 370 may not adequately address uncertainties, especially since, non-carcinogenic effects were observed at all doses tested. Weighing the conservatism of the exposure assessment to Pigment Red 3 in paints, the MOE of 370 does not cause any concern for short term non-carcinogenic effects.

Uncertainties in Evaluation of Risk to Human Health

The results from available genotoxicity of Pigment Red 3 are equivocal and the mode(s) of induction of tumours observed in rodents has not been elucidated. There is uncertainty concerning the potential of the substance to induce effects at lower doses, as non-cancer effects were observed at the lowest oral tested doses, which may lead to an underestimation of risk. The studies for short-term, subchronic and chronic exposure are available from administration via the oral route only. It is assumed that Pigment Red 3

has the same absorption rate through the oral or dermal route and the exposure causes similar systemic effects. . This is reasonable based on the limited bioavailability data reviewed which indicated potential maximum oral absorption of approximately 28%. This value is of similar magnitude to the skin absorption of its analogue.

Data were inadequate to permit quantification of exposure to Pigment Red 3 from environmental media in Canada. There is some uncertainty in the quantitative estimates of exposure from products containing Pigment Red because of the use of default factors, having varying degrees of confidence, in the ConsExpo consumer product modelling. Uncertainty is recognized for the absence of specific Pigment Red 3 dermal absorption rate and having to use the absorption rate of its analogue substance. For these reasons, confidence in the exposure assessment to Pigment Red 3 originating from consumer products is moderate, however the actual exposures are considered not to exceed the conservatively modelled consumer exposure estimates.

Conclusion

Based on the information presented in this screening assessment, it is concluded that Pigment Red 3 is not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

On the basis of carcinogenicity of Pigment Red 3, for which there may be a probability of harm at any level of exposure, it is concluded that Pigment Red 3 may be entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

It is therefore concluded that Pigment Red 3 meets the criterion in paragraph 64(c) of CEPA 1999. Additionally, Pigment Red 3 meets the criteria for persistence but does not meet the criteria for bioaccumulation as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

References

ACD/pK_aDB [Prediction Module]. 2005. Version 9.04. Toronto (ON): Advanced Chemistry Development. Available from: http://www.acdlabs.com/products/phys_chem_lab/pka/

Arnot JA, Gobas FAPC. 2003. A generic QSAR for assessing the bioaccumulation potential of organic chemicals in aquatic food webs. *QSAR Comb Sci* 22(3): 337-345.

[ASTER] Assessment Tools for the Evaluation of Risk [Internet]. 1999. Duluth (MN): US Environmental Protection Agency, Mid-Continent Ecology Division. Available from: http://www.epa.gov/med/Prods_Pubs/aster.htm Restricted access.

[ASTreat] Activated Sludge Treatment. Computer model for sewage treatment plant removal predictions [CD-ROM]. 2006. Version 1.0. Cincinnati (OH): Procter & Gamble. Available from: P&G, P.O. Box 538707, Cincinnati, OH, 45253-8707, USA.

Banerjee S, Baughan GL. 1991. Bioconcentration factors and lipid solubility. *Environ Sci Technol* 26:536-539.

Barański B, Przybojewska B, Spiechowicz E, Wszyńska K, Zimnicki J. 1992. Identification of potential carcinogenic dyes and intermediates on the basis of their genotoxicity. *Med Pr* 43:469-477 (in Polish, reviewed in Moller and Wallin 2000).

Baughman GL, Perenich TA. 1988. Fate of dyes in aquatic systems: I. Solubility and partitioning of some hydrophobic dyes and related compounds. *Environ Toxicol Chem* 7: 183-199.

Baughman GL, Weber EJ. 1994. Transformation of dyes and related compounds in anoxic sediment: kinetics and products. *Environ Sci Technol* 28(2): 267-276.

[BCFWIN] BioConcentration Factor Program for Windows [Estimation Model]. 2000. Version 2.15. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

Bertelsen SL, Hoffman AD, Gallinat CA, Elonen CM, Nichols JW. 1998. Evaluation of Log K_{OW} and tissue lipid content as predictors of chemical partitioning in fish tissues. *Environ Toxicol Chem* 17:1447-1455.

[BIBRA] British Industrial Biological Research Association. 1996. Toxicity Profile. Toluidine Red. The British Industrial Biological Research Association (BIBRA). Carshalton, Surrey, UK.

Brooke DN, Crookes MJ. 2007. Emission scenario document on transport and storage of chemicals [Internet]. Bristol (UK): Environment Agency. Product code: SCHO0407BMLK-E-P. [cited 2008 Feb 26]. Available from: <http://publications.environment-agency.gov.uk/pdf/SCHO0407BMLK-e-e.pdf>

Canada. 1999. *Canadian Environmental Protection Act, 1999*. S.C., 1999, c. 33. Canada Gazette. Part III. vol. 22, no. 3. Available from: <http://canadagazette.gc.ca/partIII/1999/g3-02203.pdf>

Canada. 2000. *Canadian Environmental Protection Act: Persistence and Bioaccumulation Regulations*, P.C. 2000-348, 23 March, 2000, SOR/2000-107, Canada Gazette. Part II, vol. 134, no. 7, p. 607-612. Available from: <http://canadagazette.gc.ca/partII/2000/20000329/pdf/g2-13407.pdf>

Canada, Dept. of the Environment, Dept. of Health. 2006. *Canadian Environmental Protection Act, 1999: Notice of intent to develop and implement measures to assess and manage the risks posed by certain substances to the health of Canadians and their environment*. Canada Gazette, Part I, vol. 140, no. 49, p.4109–4117. Available from: <http://canadagazette.gc.ca/partI/2006/20061209/pdf/g1-14049.pdf>

Canada, Dept. of the Environment, Dept. of Health. 2007a. *Canadian Environmental Protection Act, 1999: Notice of third release of technical information relevant to substances identified in the Challenge*. Canada Gazette, Part I, vol. 141, no. 33, p. 2375-2379. Available from: <http://canadagazette.gc.ca/partI/2007/20070818/pdf/g1-14133.pdf#page=7>

Canada, Dept. of the Environment. 2007b. *Canadian Environmental Protection Act, 1999: Notice with respect to Batch 3 Challenge substances*. Canada Gazette, Part I, vol. 141, no. 33, p.2379-2394. Available from: <http://canadagazette.gc.ca/partI/2007/20070818/pdf/g1-14133.pdf#page=7>

Clariant. 2007. Colorants for the Paint Industry. [cited 2007 June 3]. Available from: [http://www.clariant.com/C1256C70004EEA54/vwLookupDownloads/DP8523ED.pdf/\\$File/DP8523ED.pdf](http://www.clariant.com/C1256C70004EEA54/vwLookupDownloads/DP8523ED.pdf/$File/DP8523ED.pdf)

Collier SW, Storm JE, Bronaugh RL. 1993. Reduction of azo dyes *in vitro* percutaneous absorption. *Toxicol Appl Pharmacol* 118:73-79.

[CNS] Cosmetic Notification System – proprietary database. 2008. Ottawa (ON): Health Canada. Cited April 2008.

[CPMA] Color Pigments Manufacturers Association, Inc. 2003. Comments of the Color Pigments Manufacturers Association, Inc. on the Draft Guidance Manual for the Categorization of Organic and Inorganic Substances on Canada's Domestic Substances List ('DSL') and Environment Canada's Computer Generated Estimates and Empirical Data on Approximately 12,000 Discrete Organic Chemicals on the DSL. Letter to Danie Dubé, Existing Substances Branch, September 30, 2003.

[CTFA] Cosmetic, Toiletry and Fragrance Association. 2008. *International Cosmetic Ingredient Dictionary and Handbook*. The Cosmetic, Toiletry and Fragrance Association, Inc.

Danish EPA (Environmental Protection Agency). 1998. Survey of azo-colorants in Denmark: Consumption, use, health and environmental aspects. Ministry of Environment and Energy, Denmark.

[ECOSAR] Ecological Structural Activity Relationships [Internet]. 2004. Version 0.99h. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

El Dareer SM, Tillery KF, Hill DL. 1984. Investigations on the disposition of oral doses of some water-insoluble pigments. *Bull Environ Contam Toxicol* 32(2): 171-174.

Environment Canada. 2007. Assumptions, limitations and uncertainties of the MFTMass Flow Tool for Pigment Red 3 CAS RN 2425-85-6. Internal draft document. Gatineau (QC): Environment Canada, Existing Substances Division. Available on request.

Environment Canada. 2008a. Data for Batch 3 substances collected under the *Canadian Environmental Protection Act, 1999*, Section 71: *Notice with respect to Batch 3 Challenge substances*. Prepared by: Environment Canada, Health Canada, Existing Substances Program.

Environment Canada. 2008b. Guidance for conducting ecological assessments under CEPA 1999: science resource technical series, technical guidance module: Mass Flow Tool. Preliminary draft working document. Gatineau (QC): Environment Canada, Existing Substances Division.

Environment Canada. 2008c. Site-specific Exposure Scenario Report: CAS RN 2425-85-6. 2008-04-29. Unpublished report. Gatineau (QC): Environment Canada, Existing Substances Division.

report: CAS RN 2425-85-6. 2008-05-20. Unpublished report. Gatineau (QC): Environment Canada, Existing Substances Division.

European Commission. 1976. Council Directive of 27 July, 1976 on the approximation of the laws of the Member States relating to cosmetic products (76/768/EEC) (OJ L 262, 27.9.1976, p. 169. [accessed: 2008 June 2]. Available from: http://ec.europa.eu/enterprise/cosmetics/html/consolidated_dir.htm

Graham SL, Davis KJ. 1968. Subacute toxicity of toluidine red. *Toxicol Appl Pharmacol* 13: 388-391.

Health Canada. 1998. Exposure factors for assessing total daily intake of Priority Substances by the general population of Canada. Unpublished report, December 1998. Ottawa (ON): Health Canada, Environmental Health Directorate.

[HENRYWIN] Henry's Law Constant Program for Microsoft Windows [Estimation Model]. 2000. Version 3.10. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

Herbst W, Hunger K. 2004. Industrial organic pigments, 3rd edition. Weinheim (Germany): Wiley-VCH, Verlag GmbH & Co. KGaA. 660 p.

[IARC] International Agency for Research on Cancer. 1993. CI Pigment Red 3, IARC Monographs on the Evaluation of Carcinogenesis Risk of Chemicals to Man. Lyon, France: IARC. Vol. 57:259-266.

Jaffe EE. 1996. Pigments (Organic). In: Kroschwitz JI and Howe-Grant M (eds.). *Kirk-Othmer encyclopedia of chemical technology*, 4th ed. New York (NY): John Wiley and Sons. Vol. 19: 41-78.

Kgzuka T, Tashiro M, Sano S, Fujimoto K, Nakamura Y, Hashimoto S, Nakaminami G. 1980. Pigmented contact dermatitis from azo dyes. I. Cross-sensitivity in humans. *Contact Dermatitis* 6: 330-336.

[KOWWIN] Octanol-Water Partition Coefficient Program for Microsoft Windows [Estimation Model]. 2000. Version 1.67. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

Leroueil PR, Hong S, Mecke A, Baker JR Jr, Orr BG, Banaszak Holl MM. 2007. Nanoparticle interaction with biological membranes: does nanotechnology present a Janus face? *Acc Chem Res* 40: 335-342.

Lincke G. 2003. Molecular stacks as a common characteristic in the crystal lattice of organic pigment dyes. A contribution to the "soluble-insoluble" dichotomy of dyes and pigments from the technological point of view. *Dyes and Pigments*. 59: 1-24.

Mackay D. 1982. Correlation of bioconcentration factors. *Environ Sci Technol* 16: 274-278.

Miyagoshi M, Hayakawa Y, Nagayama T. 1983. Studies on the mutagenicity of cosmetic azo-dyes. *Eisei Kagaku* 29: 212-220 (reviewed in Møller and Wallin 2000).

[MJCDRG] Mid-Japan Contact Dermatitis Research Group. 1978. *J. Dermatol* 5:291 (cited in BIBRA 1996).

Møller P, Wallin H. 2000. Genotoxic hazards of azo pigments and other colorants related to 1-phenylazo-2-hydroxynaphthalene. *Mutat Res* 462: 13-30.

Morgan DL, Jameson CW, Mennear JH, Prejean D. 1989. 14-Day and 90-day toxicity studies of Pigment Red 3 in Fischer 344 rats and B6C3F1 mice. *Food Chem Toxicol* 27: 793-800.

Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B, Zeiger E. 1986. Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ Mutagen* 8: 1-119.

[MPBPWIN] Melting Point Boiling Point Program for Microsoft Windows [Estimation Model]. 2000. Version 1.41. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

[MSDS] Material Safety Data Sheet: Suco Red L 3750 [Internet]. 2004. Toronto (ON): BASF Canada. [cited 2007 Dec 27]. Available from: www.msdsonline.com [Restricted access].
[NCI] National Chemical Inventories [database on CD-ROM]. 2006. Columbus (OH): American Chemical Society, Chemical Abstracts Service. [cited 2006 Dec 11]. Available from: <http://www.cas.org/products/cd/nci/index.html>

[NPIRI] National Printing Ink Research Institute. 1983. Raw Materials Data Handbook. Volume 4. Pigments. P. 21. Napim (NY) (cited in BIBRA 1996).

[NPIRI] National Printing Ink Research Institute. 2000. Raw Materials Data Handbook. A reference guide to regulatory data and technical performance properties. Volume 4—Pigments, Second Edition. Woodbridge (NJ). National Printing Ink Research Institute P.62.

[NTP] National Toxicology Program. 1992. Toxicology and carcinogenesis studies of Pigment Red 3 (CAS No. 2425-85-6) in F344/N rats and B6C3F1 mice (feed studies). *Natl Toxicol Program Tech Rep Ser.* 407:1-289.

[OASIS Forecast] Optimized Approach based on Structural Indices Set [Internet]. 2005. Version 1.20. Bourgas, Bulgaria: Laboratory of Mathematical Chemistry. Available from: <http://oasis-lmc.org/?section=software>

[OECD] Organisation for Economic Co-operation and Development. 2000. Guidance document on aquatic toxicity testing of difficult substances and mixtures. OECD Environmental Health and Safety Publications Series on Testing and Assessment. Paris (FR): Environment Directorate, OECD. Number 23. [cited 2008 Feb 28]. Available from: http://www.epa.gov/oscpmont/oscpendo/pubs/ref-2_oecd_gd23_difficult_substances.pdf

[OECD] Organisation for Economic Co-operation and Development. 2004. Emission Scenario Document on textile finishing industry [Internet]. Paris (FR): OECD Environmental Directorate, Environmental Health and Safety Division. ENV/JM/MONO(2004)12, JT00166691 [cited 2008 Dec]. Available from: [http://www.olis.oecd.org/olis/2004doc.nsf/LinkTo/NT0000452A/\\$FILE/JT00166691.PDF](http://www.olis.oecd.org/olis/2004doc.nsf/LinkTo/NT0000452A/$FILE/JT00166691.PDF)

[OECD] Organisation for Economic Co-operation and Development. 2006. Draft emission scenario document on coating industry (paints, lacquers and varnishes). Draft document. Paris (FR): OECD.

[OECD] Organisation for Economic Co-operation and Development. 2007. Emission scenario document on adhesive formulation [Internet]. Final report. Paris (FR): OECD, Environment Directorate. (Series on Emission Scenario Documents). [cited 2008 Feb 26]. Available from: <http://ascouncil.org/news/adhesives/docs/EPAFormulation.pdf>

[PMRA] Pest Monitoring Management Agency. 2007. Memorandum to Pesticide Registrants. Post-categorization of substances on the Domestic Substances List: Impacts of the Challenge for Pesticide Registrants. Attachment 1: Actives, Formulants and Formulants Impurities on the Challenge List, resulting from Categorization of the DSL [Internet]. Ottawa (ON): Health Canada, Pest Monitoring Management

Agency. [cited 2008-08-01]. Available from: <http://www.pmra-arla.gc.ca/english/pdf/appregis/Memo-Post-categorization-e.pdf>

[PMRA] Pest Management Regulatory Agency. 2007a. Regulatory Note REG 2007-04: PMRA list of formulants [Internet]. Ottawa (ON): Health Canada, Pest Management Regulatory Agency. [cited 2008-08-01]. Available from: <http://www.pmra-arla.gc.ca/english/pdf/reg/reg2007-04-e.pdf>

[RIVM] Rijksinstituut voor Volksgezondheid en Milieu. 2007. Paint Fact Sheet to Assess the Risks for the Consumer. Available from: <http://www.rivm.nl/cgi-bin/htsearch?config=zoeken&words=fact+sheet&method=and&restrict=>

[SCC] Scientific Committee on Cosmetology. 1988. Reports of the Scientific Committee on Cosmetology. 7th Series. Environmental and Quality of Life Report. Commission of the European communities. EUR 11303 EN (cited in BIBRA 1996).

[SPIN] Substances in Products in Nordic Countries [database on the Internet]. 2006. Copenhagen (DK): Nordic Council of Ministers. [cited 2006 Dec 11]. Available from: <http://195.215.251.229/Dotnetnuke/Home/tabid/58/Default.aspx>

Study Submission. 2007a. Unpublished confidential study submitted to Environment Canada, Existing Substances Division under the Chemical Management Plan Challenge initiative. Available as Robust Study Summary, Identification No.: 13365Submission011. (See Appendix 1A).

Study Submission. 2007b. Unpublished confidential study submitted to Environment Canada, Existing Substances Division under the Chemical Management Plan Challenge initiative. Available as Robust Study Summary, Identification No.: 13365Submission012. (See Appendix 1B).

Study Submission. 2007c. Unpublished confidential study submitted to Environment Canada, Existing Substances Division under the Chemical Management Plan Challenge initiative. Available as Robust Study Summary, Identification No.: 13365Submission013. (See Appendix 1C).

Study Submission. 2007d. Unpublished confidential study submitted to Environment Canada, Existing Substances Division under the Chemical Management Plan Challenge initiative. Available as Robust Study Summary, Identification No.: 13365Submission014. (See Appendix 1D).

Study Submission. 2007e. Unpublished confidential study submitted to Environment Canada, Existing Substances Division under the Chemical Management Plan Challenge initiative. Available as Robust Study Summary, Identification No.: 13365Submission016. (See Appendix 1E).

Study Submission. 2007f. Unpublished confidential study submitted to Environment Canada, Existing Substances Division under the Chemical Management Plan Challenge initiative. Available as Robust Study Summary, Identification No.: 13365Submission017. (See Appendix 1F).

Study Submission. 2007g. Unpublished confidential study submitted to Environment Canada, Existing Substances Division under the Chemical Management Plan Challenge initiative. Available as Robust Study Summary, Identification No.: 13365Submission020. (See Appendix 1G).

Study Submission. 2007h. Unpublished confidential study submitted to Environment Canada, Existing Substances Division under the Chemical Management Plan Challenge initiative. Available as Robust Study Summary, Identification No.: 13365Submission015. (See Appendix 1H).

Sugai T, Takahashi Y, Takagi T. 1977. Pigmented cosmetic dermatitis and coal tar dyes. *Contact Derm* 3: 249-256 (cited in IARC 1993).

Tsuda S, Matsusaka N, Madarame H, Ueno S, Susa N, Ishida K, Kawamura N, Sekihashi K, Sasaki YF. 2000. The comet assay in eight mouse organs: results with 24 azo compounds. *Mutat Res* 465: 11-26.

- Van der Zee FP. 2002. Anaerobic azo dye reduction. Doctoral Thesis, Wageningen University. Wageningen, The Netherlands. pp 142.
- Weber EJ, Adams RL. 1995. Chemical- and sediment-mediated reduction of the azo dye Disperse Blue 79. *Environ Sci Technol* 29: 1163-1170.
- Weber EJ, Wolfe NL. 1987. Kinetic studies of the reduction of aromatic azo compounds in anaerobic sediment/water systems. *Environ Toxicol Chem* 6:911-919.
- Whitaker A. 1978. Crystal structure analysis of azo pigments involving β -Naphthol: A review. *J. Soc. Dyers Col. (Communications)*. 431-435.
- Wiesner MR, Lowry GV, Alvarez P, Dionysiou D, Biswas P. 2006. Assessing the risks of manufactured nanomaterials. *Environ Sci Technol* 40:4336-4345.
- [WSKOWWIN] Water Solubility for Organic Compounds Program for Microsoft Windows [Estimation Model]. 2000. Version 1.41 Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm
- Yen CC, Perenich TA, Baughman GL. 1991. Fate of commercial disperse dyes in sediments. *Environ Toxicol Chem* 10:1009-1017.

Appendix 1. Robust study summaries

A. Evaluation of experimental data using Kollig's approach*

Item	Weight	Response	Mark
Reference: Study Submission 2007a.13365Submission011. Water Solubility Following ETAD Method			
Test substance: CAS RN: 2425-85-6, Pigment Red 3			
Could you repeat the experiment with available information?	5	Yes	5
Is a clear objective stated?	1	Yes	1
Is water quality characterized or identified (distilled or deionized)?	2	No	2
Are the results presented in detail, clearly and understandably?	3	Yes	3
Are the data from a primary source and not from a referenced article?	3	Yes	3
Was the chemical tested at concentrations below its water solubility?	5	N/A	
Were particulates absent?	2	Yes	2
Was a reference chemical of known constant tested?	3	No	0
Were other fate processes considered?	5	N/A	
Was a control (blank) run?	3	Yes	1.5
Was temperature kept constant?	5	Assumed	5
Was the experiment done near room temperature (15–30°C)?	3	Yes	3
Is the purity of the test chemical reported (> 98%)?	3	Yes	3
Was the chemical's identity proven?	3	Yes	3
Is the source of the chemical reported?	1	No	0
Results: ($\bar{X} \pm SE$)			
		Solubility = 3.3ug/L	
Score:	31.5/37=85%		
Degree of reliability**	High		
Comments			

* Kollig, H.P. 1988. Criteria for evaluating the reliability of literature data on environmental process constants. Toxicol. Environ. Chem. 17: 287-311.

** The reliability code for ecotoxicological studies of DSL categorization is used.

B. Evaluation of experimental data using Kollig's approach*

Item	Weight	Response	Mark
Reference: Study Submission 2007b. 13365Submission012. Octanol Solubility Following ETAD Method			
Test substance: CAS RN: 2425-85-6, Pigment Red 3			
Could you repeat the experiment with available information?	5	Yes	5
Is a clear objective stated?	1	Yes	1
Is water quality characterized or identified (distilled or deionized)?	2	No	2
Are the results presented in detail, clearly and understandably?	3	Yes	3
Are the data from a primary source and not from a referenced article?	3	Yes	3
Was the chemical tested at concentrations below its water solubility?	5	N/A	
Were particulates absent?	2	Yes	2
Was a reference chemical of known constant tested?	3	No	0
Were other fate processes considered?	5	N/A	
Was a control (blank) run?	3	Yes	1.5
Was temperature kept constant?	5	Assumed	5
Was the experiment done near room temperature (15–30°C)?	3	Yes	3
Is the purity of the test chemical reported (> 98%)?	3	Yes	3
Was the chemical's identity proven?	3	Yes	3
Is the source of the chemical reported?	1	No	0
Results: ($\bar{X} \pm SE$)			
		Solubility = 17.9 mg/L	
Score:	31.5/37=85%		
Degree of reliability**	High		
Comments			

* Kollig, H.P. 1988. Criteria for evaluating the reliability of literature data on environmental process constants. Toxicol. Environ. Chem. 17: 287-311.

** The reliability code for ecotoxicological studies of DSL categorization is used.

C. Evaluation of experimental data using Kollig's approach*

Item	Weight	Response	Mark
Reference: Study Submission 2007c. 13365Submission013. Water Solubility Following ETAD Method			
Test substance: CAS RN: 2814-77-9, Pigment Red 4			
Could you repeat the experiment with available information?	5	Yes	5
Is a clear objective stated?	1	Yes	1
Is water quality characterized or identified (distilled or deionized)?	2	No	2
Are the results presented in detail, clearly and understandably?	3	Yes	3
Are the data from a primary source and not from a referenced article?	3	Yes	3
Was the chemical tested at concentrations below its water solubility?	5	N/A	
Were particulates absent?	2	Yes	2
Was a reference chemical of known constant tested?	3	No	0
Were other fate processes considered?	5	N/A	
Was a control (blank) run?	3	Yes	1.5
Was temperature kept constant?	5	Assumed	5
Was the experiment done near room temperature (15–30°C)?	3	Yes	3
Is the purity of the test chemical reported (> 98%)?	3	Yes	3
Was the chemical's identity proven?	3	Yes	3
Is the source of the chemical reported?	1	No	0
Results: ($\bar{X} \pm SE$)			
		Solubility = 3.3 ug/L	
Score:	31.5/37=85%		
Degree of reliability**	High		
Comments			

* Kollig, H.P. 1988. Criteria for evaluating the reliability of literature data on environmental process constants. Toxicol. Environ. Chem. 17: 287-311.

** The reliability code for ecotoxicological studies of DSL categorization is used.

D. Evaluation of experimental data using Kollig's approach*

Item	Weight	Response	Mark
Reference: Study Submission 2007d. 13365Submission014. Octanol Solubility Following ETAD Method			
Test substance: CAS RN: 2814-77-9, Pigment Red 4			
Could you repeat the experiment with available information?	5	Yes	5
Is a clear objective stated?	1	Yes	1
Is water quality characterized or identified (distilled or deionized)?	2	No	2
Are the results presented in detail, clearly and understandably?	3	Yes	3
Are the data from a primary source and not from a referenced article?	3	Yes	3
Was the chemical tested at concentrations below its water solubility?	5	N/A	
Were particulates absent?	2	Yes	2
Was a reference chemical of known constant tested?	3	No	0
Were other fate processes considered?	5	N/A	
Was a control (blank) run?	3	Yes	1.5
Was temperature kept constant?	5	Assumed	5
Was the experiment done near room temperature (15–30°C)?	3	Yes	3
Is the purity of the test chemical reported (> 98%)?	3	Yes	3
Was the chemical's identity proven?	3	Yes	3
Is the source of the chemical reported?	1	No	0
Results: ($\bar{X} \pm SE$)			
		Solubility = 9.4 mg/L	
Score:	31.5/37=85%		
Degree of reliability**	High		
Comments			

* Kollig, H.P. 1988. Criteria for evaluating the reliability of literature data on environmental process constants. Toxicol. Environ. Chem. 17: 287-311.

** The reliability code for ecotoxicological studies of DSL categorization is used.

E. Evaluation of experimental data using Kollig's approach*

Item	Weight	Response	Mark
Reference: Study Submission 2007e. 13365Submission016. Water Solubility Following ETAD Method			
Test substance: CAS RN: 3468-63-1, Pigment Orange 5			
Could you repeat the experiment with available information?	5	Yes	5
Is a clear objective stated?	1	Yes	1
Is water quality characterized or identified (distilled or deionized)?	2	No	2
Are the results presented in detail, clearly and understandably?	3	Yes	3
Are the data from a primary source and not from a referenced article?	3	Yes	3
Was the chemical tested at concentrations below its water solubility?	5	N/A	
Were particulates absent?	2	Yes	2
Was a reference chemical of known constant tested?	3	No	0
Were other fate processes considered?	5	N/A	
Was a control (blank) run?	3	Yes	1.5
Was temperature kept constant?	5	Assumed	5
Was the experiment done near room temperature (15–30°C)?	3	Yes	3
Is the purity of the test chemical reported (> 98%)?	3	Yes	3
Was the chemical's identity proven?	3	Yes	3
Is the source of the chemical reported?	1	No	0
Results: ($\bar{X} \pm SE$)			
		Solubility = 6.8 ug/L	
Score:	31.5/37=85%		
Degree of reliability**	High		
Comments			

* Kollig, H.P. 1988. Criteria for evaluating the reliability of literature data on environmental process constants. Toxicol. Environ. Chem. 17: 287-311.

** The reliability code for ecotoxicological studies of DSL categorization is used.

F. Evaluation of experimental data using Kollig's approach*

Item	Weight	Response	Mark
Reference: Study Submission 2007f. 13365Submission017. Octanol Solubility Following ETAD Method			
Test substance: CAS RN: 3468-63-1, Pigment Orange 5			
Could you repeat the experiment with available information?	5	Yes	5
Is a clear objective stated?	1	Yes	1
Is water quality characterized or identified (distilled or deionized)?	2	No	2
Are the results presented in detail, clearly and understandably?	3	Yes	3
Are the data from a primary source and not from a referenced article?	3	Yes	3
Was the chemical tested at concentrations below its water solubility?	5	N/A	
Were particulates absent?	2	Yes	2
Was a reference chemical of known constant tested?	3	No	0
Were other fate processes considered?	5	N/A	
Was a control (blank) run?	3	Yes	1.5
Was temperature kept constant?	5	Assumed	5
Was the experiment done near room temperature (15–30°C)?	3	Yes	3
Is the purity of the test chemical reported (> 98%)?	3	Yes	3
Was the chemical's identity proven?	3	Yes	3
Is the source of the chemical reported?	1	No	0
Results: ($\bar{X} \pm SE$)			
		Solubility = 1.76 mg/L	
Score:	31.5/37=85%		
Degree of reliability**	High		
Comments			

* Kollig, H.P. 1988. Criteria for evaluating the reliability of literature data on environmental process constants. Toxicol. Environ. Chem. 17: 287-311.

** The reliability code for ecotoxicological studies of DSL categorization is used.

G. Robust Study Summary

Robust Study Summary Form: Aquatic iT					
No	Item	Weight	Yes/No		
1	Study Submission 2007g. 13365Submission020. Acute Immobilisation Test (Static, 48h) to <i>Daphnia magna</i> , Limit Test				
2	Substance identity: CAS RN	n/a	Y	2425-85-6	Chemical Abstracts Service Registry Number.
3	Substance identity: chemical name(s)	n/a	Y	Pigment Red 3	At least one chemical name from a recognized nomenclature.
4	Chemical composition of the substance	2		n/a	Yes or No. Chemical composition (%) of the substance (major and secondary components, by-products, impurities). Especially important for UVCBs and polymers. May be considered as "non-applicable" if the test substance is a discrete high-purity chemical (see item 5).
5	Chemical purity	1	Y	98.20%	Yes or No. Purity may be reported as % and/or chemical grade designations (e.g. A.C.S., Reagent, etc.). May be not applicable for some UVCBs (e.g. CAS 128683-25-0 - crude oil; CAS 65996-72-7 - steelmaking dust; etc).
6	Persistence/stability of test substance in aquatic solution reported?	1	Y		Yes or No. Information on whether the substance is stable or unstable (i.e. volatile, hydrolysable, photodegradable, polymerizable, readily biodegradable, etc.) in water.
Method					
7	Reference	1	Y		Yes or No. Reference in respect to the method used.
8	OECD, EU, national, or other standard method?	3	Y	OECD 202	Yes or No.
9	Justification of the method/protocol if a standard method was not used	2		n/a	Yes or No. When "Yes" method justification (which is not a synonym of the "method description") should be provided. Not applicable, if a standard protocol (see item 8) was used.
10	GLP (Good Laboratory Practice)	3	Y		Yes or No. "Yes" - whenever GLP was applied. "No" - if the study was conducted after 1997 and GLP was not implemented. If the study completed before 1997 and GLP was not implemented, the item can be considered as not applicable.
Test organism					
11	Organism identity: name	n/a	Y	<i>Daphnia magna</i> STRAUS	Names (common and/or scientific) as reported in the study.
12	Latin or both Latin & common names reported?	1	Y		Yes or No.
13	Life cycle age / stage of test organis	1	Y		Yes or No. Item may not be negatively answered if other items (e.g. item 15) give indirect, but clear information on the age or stage of the organism (if the weight/size of the specific fish species is given, an assumption on the life stage can be easily made).
14	Length and/or weight	1	Y		Yes or No. Not applicable in some cases (e.g. for algae or very small invertebrates such as <i>Daphnia magna</i>).
15	Sex	1		n/a	Yes or No. Not applicable in some cases (e.g. algae). If the item is applicable for an organism, but the organism is very young and small, for example 1–3 cm long fathead minnow or rainbow trout (e.g. see OECD Guideline No. 203), this item can also be considered as non-applicable.
16	Number of organisms per replicate	1	Y	5	Yes or No. Specify number of organisms per replicate
17	Organism loading rate	1	N		Yes or No. Specify loading rate for the organisms (e.g. 0.8 g/L).

18	Food type and feeding periods during the acclimation period	1	Y		Yes or No.
Test design / conditions					
19	Test type (acute or chronic)	n/a	Y	Acute	Yes or No. Specify test type.
20	Experiment type (laboratory or field)	n/a	Y	Laboratory	Yes or No. Specify test type.
21	Exposure pathways (food, water, both)	n/a	Y	Water	Yes or No. Specify exposure pathways.
22	Exposure duration	n/a	Y	48h	Yes or No. Specify exposure duration.
23	Negative or positive controls (specify)	1	Y	Positive & Negative	Yes or No. Specify which controls were used.
24	Number of replicates (including controls)	1	Y	4	Yes or No. Specify number of replicates.
25	Nominal concentrations reported?	1	N		Yes or No. Specify number of nominal concentrations.
26	Measured concentrations reported?	3	Y	Reported as DOC	Yes or No. May be considered as not applicable if measured concentrations are reported.
27	Food type and feeding periods during the long-term tests	1		n/a	Yes or No. Not applicable for acute tests as organisms are not normally fed in short-term tests.
28	Were concentrations measured periodically (especially in the chronic test)?	1	Y	2 measurements	Yes or No. When answered "Yes": chronic tests - at least three measurements; acute tests - at least two measurements (in both cases, actual concentrations at the end of the test have to be presented).
29	Were the exposure media conditions relevant to the particular chemical reported? (e.g., for the metal toxicity - pH, DOC/TOC, water hardness, temperature)	3	Y		Yes or No.
30	Photoperiod and light intensity	1	Y		Yes or No.
31	Stock and test solution preparation	1	Y		Yes or No.
32	Was solubilizer/emulsifier used, if the chemical was poorly soluble or unstable?	1	N		Yes or No. Applicable for poorly soluble / unstable substances only, especially when toxicity value is above the chemical's water solubility.
33	If solubilizer/emulsifier was used, was its concentration reported?	1		n/a	Yes or No.
34	If solubilizer/emulsifier was used, was its ecotoxicity reported?	1		n/a	Yes or No. It is allowed to present toxicity values from other similar tests.
35	Analytical monitoring intervals	1	Y		Yes or No.
36	Statistical methods used	1	Y		Yes or No.
Information relevant to the data quality					
37	Was the endpoint directly caused by the chemical's toxicity, not by organism's health (e.g. when mortality in the control >10%) or physical effects (e.g. "shading effect")?	n/a	Y		Yes or No. When answered "No", submit all the considerations/concerns in the "Comments" box below; most likely, the study will be rejected.
38	Was the test organism relevant to the Canadian environment?	3	Y		Yes or No.
39	Were the test conditions (pH, temperature, DO, etc.) typical for the test organism?	1	Y		Yes or No.
40	Does system type and design (static, semi-static, flow-through; sealed or open; etc.) correspond to the substance's properties and organism's	2	Y		Yes or No. "No" - when, for example, the chemical was volatile, and open (not sealed) static-system tanks were used.

	nature/habits?				
41	Was pH of the test water within the range typical for the Canadian environment (6 to 9)?	1	Y		Yes or No. Specify actual pH range.
42	Was temperature of the test water within the range typical for the Canadian environment (5 to 27°C)?	1	Y		Yes or No. Specify actual temperature range.
43	Was toxicity value below the chemical's water solubility?	3		n/a. Was tested at saturation and no effect was observed.	Yes or No. Experimental WS results are preferred over predicted WS data. The difference between WS and toxicity value is considered to be negligible when it is within one order of magnitude, and if beyond that, the difference is considered as meaningful. The item is not applicable if WAF (Water Accommodated Fractions) protocol has been used.
Results					
44	Toxicity values (specify endpoint and value)	n/a	n/a	No effect at DOC=2.6mg/L	Specify endpoint and value (e.g. 48-h LC ₅₀ =70 mg/L)
45	Other endpoints (e.g., BCF/BAF, LOEC/NOEC) reported? (specify)	n/a	N		Yes or No. Specify endpoint and value (e.g. 28-d NOEC=70 mg/L; BCF=1200 L/kg)
46	Other adverse effects (e.g., carcinogenicity, mutagenicity) reported?	n/a	N		Yes or No. Specify other adverse effects (if any)
47	Score: ... %	92.1			
48	EC Reliability code:	1			
49	Reliability category (high, satisfactory, low):	High Confidence			
50	Comments	<p>The DOC of the pigment is 0.6mg/L at t=0hr and t=48hr. The TOC of the pigment is 66.4%. The average concentration of the pigment in water can be calculated based on $[DOC] = [DOC \text{ of pigment} \times \text{purity of pigment} / \text{fraction OC of pigment}] = 0.6 \text{ mg DOC/L} \times 0.982 / 0.644 = 0.9 \text{ mg/L}$ pigment. The pigment's water solubility is 0.0033 mg/L (experimental). i.e. the "saturation" value dramatically exceeds the water solubility value; therefore it is assumed that 0.9mg/L corresponds to a mixture of pigment particles and a small dissolved fraction. The stock solution was shaken for 24 hours, followed by centrifugation. There is uncertainty that the maximum solubility was achieved, as the solution was only shaken for 24 hours and the temperature was not elevated. The OECD Guidance for aquatic toxicity testing of difficult substances also indicates that the concentration measured is typically less than the water solubility if it is saturated. The study demonstrates that the pigment particles and dissolved pigment caused no effects on <i>Daphnia magna</i>.</p>			

H. Robust Study Summary

Robust Study Summary Form: Aquatic iT					
No	Item	Weight	Yes/No		
1	Reference. Study Submission 2007h. 13365Submission015. Acute Immobilisation Test (Static, 48h) to <i>Daphnia magna</i> , Limit Test				
2	Substance identity: CAS RN	n/a	Y	3468-63-1	Chemical Abstracts Service Registry Number.
3	Substance identity: chemical name(s)	n/a	Y	Pigment Orange 5	At least one chemical name from a recognized nomenclature.
4	Chemical composition of the substance	2		n/a	Yes or No. Chemical composition (%) of the substance (major and secondary components, by-products, impurities). Especially important for UVCBs and polymers. May be considered as "non-applicable" if the test substance is a discrete high-purity chemical (see item 5).
5	Chemical purity	1	Y	98.78%	Yes or No. Purity may be reported as % and/or chemical grade designations (e.g. A.C.S., Reagent, etc.). May be not applicable for some UVCBs (e.g. CAS 128683-25-0 - crude oil; CAS 65996-72-7 - steelmaking dust; etc).
6	Persistence/stability of test substance in aquatic solution reported?	1	Y		Yes or No. Information on whether the substance is stable or unstable (i.e. volatile, hydrolysable, photodegradable, polymerizable, readily biodegradable, etc.) in water.
Method					
7	Reference	1	Y		Yes or No. Reference in respect to the method used.
8	OECD, EU, national, or other standard method?	3	Y	OECD 202	Yes or No.
9	Justification of the method/protocol if a standard method was not used	2		n/a	Yes or No. When "Yes", method justification (which is not a synonym of the "method description") should be provided. Not applicable, if a standard protocol (see item 8) was used.
10	GLP (Good Laboratory Practice)	3	Y		Yes or No. "Yes" - whenever GLP was applied. "No" - if the study was conducted after 1997 and GLP was not implemented. If the study was completed before 1997 and GLP was not implemented, the item can be considered as not applicable.
Test organism					
11	Organism identity: name	n/a	Y	<i>Daphnia magna</i> STRAUS	Names (common and/or scientific) as reported in the study.
12	Latin or both Latin & common names reported?	1	Y		Yes or No.
13	Life cycle age / stage of test organism	1	Y		Yes or No. Item may not be negatively answered if other items (e.g. item 15) give indirect, but clear information on the age or stage of the organism (for example, if the weight/size of the specific fish species is given, an assumption on the life stage can be easily made).
14	Length and/or weight	1	Y		Yes or No. Not applicable in some cases (e.g. for algae or very small invertebrates such as <i>Daphnia magna</i>).
15	Sex	1		n/a	Yes or No. Not applicable in some cases (e.g. algae). If the item is applicable for an organism, but the organism is very young and small, for example 1–3 cm long fathead minnow or rainbow trout (e.g. see OECD Guideline No. 203), this item can also be considered as non-applicable.
16	Number of organisms per replicate	1	Y	5	Yes or No. Specify number of organisms per replicate
17	Organism loading rate	1	N		Yes or No. Specify loading rate for the organisms (e.g. 0.8 g/L).
18	Food type and feeding periods	1	Y		Yes or No.

	during the acclimation period				
Test design / conditions					
19	Test type (acute or chronic)	n/a	Y	Acute	Yes or No. Specify test type.
20	Experiment type (laboratory or field)	n/a	Y	Laboratory	Yes or No. Specify test type.
21	Exposure pathways (food, water, both)	n/a	Y	Water	Yes or No. Specify exposure pathways.
22	Exposure duration	n/a	Y	48h	Yes or No. Specify exposure duration.
23	Negative or positive controls (specify)	1	Y	Positive & Negative	Yes or No. Specify which controls were used.
24	Number of replicates (including controls)	1	Y	4	Yes or No. Specify number of replicates.
25	Nominal concentrations reported?	1	N		Yes or No. Specify number of nominal concentrations.
26	Measured concentrations reported?	3	Y	Reported as DOC	Yes or No. May be considered as not applicable if measured concentrations are reported.
27	Food type and feeding periods during the long-term tests	1		n/a	Yes or No. Not applicable for acute tests as organisms are not normally fed in short-term tests.
28	Were concentrations measured periodically (especially in the chronic test)?	1	Y	2 measurements	Yes or No. When answered "Yes": chronic tests - at least three measurements; acute tests - at least two measurements (in both cases, actual concentrations at the end of the test have to be presented).
29	Were the exposure media conditions relevant to the particular chemical reported? (e.g., for the metal toxicity - pH, DOC/TOC, water hardness, temperature)	3	Y		Yes or No.
30	Photoperiod and light intensity	1	Y		Yes or No.
31	Stock and test solution preparation	1	Y		Yes or No.
32	Was solubilizer/emulsifier used, if the chemical was poorly soluble or unstable?	1	N		Yes or No. Applicable for poorly soluble / unstable substances only, especially when toxicity value is above the chemical's water solubility.
33	If solubilizer/emulsifier was used, was its concentration reported?	1		n/a	Yes or No.
34	If solubilizer/emulsifier was used, was its ecotoxicity reported?	1		n/a	Yes or No. It is allowed to present toxicity values from other similar tests.
35	Analytical monitoring intervals	1	Y		Yes or No.
36	Statistical methods used	1	Y		Yes or No.
Information relevant to the data quality					
37	Was the endpoint directly caused by the chemical's toxicity, not by organism's health (e.g. when mortality in the control >10%) or physical effects (e.g. "shading effect")?	n/a	Y		Yes or No. When answered "No", submit all the considerations/concerns in the "Comments" box below; most likely, the study will be rejected.
38	Was the test organism relevant to the Canadian environment?	3	Y		Yes or No.
39	Were the test conditions (pH, temperature, DO, etc.) typical for the test organism?	1	Y		Yes or No.
40	Does system type and design (static, semi-static, flow-through; sealed or open; etc.) correspond to the substance's properties and organism's	2	Y		Yes or No. "No" - when, for example, the chemical was volatile, and open (not sealed) static-system tanks were used.

	nature/habits?				
41	Was pH of the test water within the range typical for the Canadian environment (6 to 9)?	1	Y		Yes or No. Specify actual pH range.
42	Was temperature of the test water within the range typical for the Canadian environment (5 to 27°C)?	1	Y		Yes or No. Specify actual temperature range.
43	Was toxicity value below the chemical's water solubility?	3		n/a. Was tested at saturation and no effect was observed.	Yes or No. Experimental WS results are preferred over predicted WS data. The difference between WS and toxicity value is considered as negligible when it is within one order of magnitude, and if beyond that, the difference is considered as meaningful. The item is not applicable if WAF (Water Accommodated Fractions) protocol has been used.
Results					
44	Toxicity values (specify endpoint and value)	n/a	n/a	No effect at DOC=2.2mg/L	Specify endpoint and value (e.g. 48-h LC ₅₀ =70 mg/L)
45	Other endpoints reported - e.g. BCF/BAF, LOEC/NOEC (specify)?	n/a	N		Yes or No. Specify endpoint and value (e.g. 28-d NOEC=70 mg/L; BCF=1200 L/kg)
46	Other adverse effects (e.g. carcinogenicity, mutagenicity) reported?	n/a	N		Yes or No. Specify other adverse effects (if any)
47	Score: ... %	92.1			
48	EC Reliability code:	1			
49	Reliability category (high, satisfactory, low):	High Confidence			
50	Comments	<p>The average DOC of the pigment is 0.9mg/L. The TOC of the pigment is 56.81%. The average concentration of the pigment in water can be calculated based on $[\text{DOC}] = [\text{DOC of pigment} \times \text{purity of pigment} / \text{fraction OC of pigment}] = 0.9 \text{ mg DOC/L} \times 0.988 / 0.568 = 1.6 \text{ mg/L pigment}$. The pigment's water solubility is only 0.0068 mg/L (experimental) i.e. the "saturation" value dramatically exceeds the water solubility value; therefore it is assumed that 0.9mg/L corresponds to a mixture of pigment particles and a small dissolved fraction. The stock solution was shaken for 24 hours and filtered with a 0.45-micrometer filter, however, the average particle size of Pigment Orange 5 is only 285nm. This indicates that the filter would not remove the particles, which confirms the above conclusion that the pigment in the test solution is represented by the pigment particles and the water-soluble fraction of the substance. There is uncertainty that the maximum solubility was achieved, as the solution was only shaken for 24 hours and the temperature was not elevated. The OECD Guidance for aquatic toxicity testing of difficult substances also indicates that the concentration measured will typically be less than the water solubility if it is saturated. The study demonstrates that the pigment particles and dissolved pigment caused no effects on <i>Daphnia magna</i>.</p>			

Appendix 2. Upper-bounding estimates of exposure to Pigment Red 3 in consumer products using ConsExpo 4.1 (RIVM 2006a)

Type of Products (% Pigment Red 3)	Assumptions ¹ : Body weight of 70.9 kg for an adult; Inhalation rate of 16.2 m ³ /day; Room temperature of 20°C (RIVM 2007); Dermal uptake fraction of 0.26 (Collier et al. 1993)	Exposure Estimate* (mg/kg-bw)
Solvent rich paint (RIVM 2007) (<15 %)²	Inhalation: Exposure to vapour by evaporation from increasing area for the brushing or rolling Exposure frequency of 1/year, exposure duration of 132 minutes, applied amount of 1 kg, release area of 10 m², room volume of 20 m³, ventilation rate of 0.6 hr⁻¹, application duration of 120 minutes, molecular weight matrix of 300 g/mol, mass transfer rate of 2.15x10³ m/min (Langmuir), and uptake fraction of 1. Dermal: Direct dermal contact with product - constant rate Contact rate of 30 mg/min and release duration of 120 min.	Inhalation: 1.36x10 ⁻⁸ Dermal: Internal: 1.98
High solid paint (RIVM 2007) (<15%)²	Inhalation: Exposure to vapour by evaporation from increasing area for the brushing or rolling Same assumptions as in solvent rich paint except for the following assumptions: applied amount of 1.3 kg, molecular weight matrix of 550 g/mol, and mass transfer rate of 2.15x10³ m/min (Langmuir). Dermal: Same as for high solvent paint	Inhalation: 2.23x10 ⁻⁸ Dermal: Internal: 1.98
Aerosol paint (RIVM 2007) (<10%)³	Inhalation: Exposure to spray, spraying away from exposed person Exposure duration of 20 minutes, room volume of 34 m³, ventilation rate of 1.5 hr⁻¹, mass generation rate of 0.33 g/sec, spray duration of 15 minutes, airborne fraction of 1, weight fraction non-volatile of 0.3, density non-volatile of 1.5 g/cm³, room height of 2.25 m, inhalation cut-off diameter of 15 µm, and non-respirable uptake fraction of 1. Dermal: Direct dermal contact with product - constant rate Contact rate of 100 mg/min and release duration of 15 min.	Inhalation: 0.119 Dermal: Internal: 0.55
Nail polish (RIVM 2006b) (< 0.1%)⁴	Inhalation: Exposure to vapour by evaporation Exposure frequency of 156/year, exposure duration of 5 minutes, applied amount of 0.25 g, release area of 19 cm², room volume of 1m³, ventilation rate of 1 hr⁻¹, application duration of 5 minutes, molecular weight matrix of 124 g/mol, mass transfer rate of 2.15x10³ m/min (Langmuir), and uptake fraction of 1. Dermal: Direct dermal contact with product – instant application Exposed area of 4 cm² and applied amount of 0.05 g.	Inhalation: 1.35x10 ⁻¹² Dermal: Internal: 1.83x10 ⁻⁴
Adult hand washing with a soap bar (RIVM 2006b)	Dermal: Direct dermal contact with product – instant application Exposure frequency of 1820/year (5 – 6/day), exposed area of 860 cm², contact rate of 30 mg/min, release duration of 120 min, applied amount of 0.8 g⁴, and estimated dilution factor of 3 (wetting hands).	Dermal: 4.4 x 10 ⁻³

Type of Products (% Pigment Red 3) ($< 0.1\%$) ⁴	Assumptions¹ : Body weight of 70.9 kg for an adult; Inhalation rate of 16.2 m ³ /day; Room temperature of 20°C (RIVM 2007); Dermal uptake fraction of 0.26 (Collier et al. 1993)	Exposure Estimate* (mg/kg-bw)
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¹ Health Canada 1998, unless otherwise indicated.

² Environment Canada 2008a

³ RC Industry. 2004-12-16. Rust-Oleum High Performance Industrial Enamel Topcoats. Material Safety Data Sheet [Internet]. 706 S. 10th St. Petersburg, IN, 47567, USA. Available from: <http://www.rcindustry.com/misc/MSDS.PDF>

⁴ [CNS] Cosmetic Notification System [Proprietary database]. 2008. Available to Cosmetics Division, Health Canada.

Appendix* Exposure estimate for a single use of a product except for a soap bar where daily exposure is estimated.

Appendix 3. Summary of health effects information for Pigment Red 3

Endpoint	Lowest effect levels ¹ /Results
Experimental animals and cells	
Acute toxicity	<p>Oral LD₅₀ (rat) > 5 g/kg-bw in rat (strain not stated) (NPIRI 1983)</p> <p>Dermal Irritation: No overt irritation was observed in the intact and abraded skin of rabbits in covered contact with 500 mg undiluted C.I. Pigment 3 (SCC 1988).</p> <p>No inhalation studies identified.</p>
Short-term repeated-dose toxicity	<p>Lowest oral LOAEL: 738 mg/kg-bw/day (6000 ppm in diet) was identified based on hematological effects (decrease in hemoglobin concentration) in Fisher 344 female rats with treatment of C.I. Pigment Red 3 in diet (0 ppm, 6000 ppm, 12 500 ppm, 25 000 ppm, 50 000 ppm, 100 000 ppm) for 14 days (Morgan et al.1989).</p> <p>Other oral studies: Significantly decreased concentrations of hematocrit, hemoglobin and erythrocyte were observed at the lowest dose about 1048 mg/kg-bw/day (0.6% in diet) in B6C3F1 male mice fed with C.I. Pigment Red 3 in diet (0 ppm, 6000 ppm, 12 500 ppm, 25 000 ppm, 50 000 ppm, 100 000 ppm) for 14 days (Morgan et al.1989).</p> <p>No inhalation or dermal studies identified.</p>
Subchronic toxicity	<p>Lowest oral LOAEL: 183 mg/kg-bw/day (3000 ppm in diet) was identified based on increased liver to body weight ratio, histopathological effects (hyperplasia in bone marrow, congestion, hematopoietic cell proliferation and pigment in spleen) and hematological effects (decrease in hematocrit concentration) in Fisher 344 male rats (10 animals/group) with treatment of C.I. Pigment Red 3 in diet (0 ppm, 3000 ppm, 6000 ppm, 12 500 ppm, 25 000 ppm, 50 000 ppm) for 90 days. Clear dose-response relationships were observed for these effects (Morgan et al.1989; NTP 1992).</p> <p>Other oral studies: Significant increase of haematopoietic cell proliferation in spleen was observed at the lowest dose 502-666 mg/kg-bw/day (3000 ppm in diet) in B6C3F1 mice (10 animals/group) fed with C.I. Pigment Red 3 in diet (0 ppm, 3000 ppm, 6000 ppm, 12 500 ppm, 25 000 ppm, 50 000 ppm) for 90 days. Severed lesions were observed at higher concentrations (Morgan et al.1989; NTP 1992).</p> <p>Significant increase of liver to body weight ratio were seen in male Osborne-Mendel rats fed with diet containing 0 ppm, 2500 ppm C.I. Pigment Red 3 for 90 days; no effects were observed on mortality, growth or hematology (Graham & Davis 1968).</p> <p>No inhalation or dermal studies identified.</p>
Chronic toxicity/carcinogenicity	<p>Oral carcinogenicity in rats: Groups of 50 male and female Fisher 344 rats were fed diets containing 0 ppm, 6000 ppm, 12 500 ppm, 25 000 ppm C.I. Pigment Red 3 for 2 years. In male rats, the incidence of benign adrenal pheochromocytomas was significantly increased in mid- and high-dose group compared to control (22/50, 29/50, 35/50, 34/50 for 0, 6000, 12 500, 25 000 ppm, respectively; p=0.004, logistic regression trend test). In female rats, hepatocellular adenoma occurred in a positive trend with significant increased incidence in the high dose (0/50, 0/50, 1/50, 10/50 for 0, 6000, 12 500, 25 000 ppm, respectively; p=0.001, logistic regression trend test). Other neoplastic effects included marginally significant increases of incidence of squamous cell papilloma or carcinoma in skin at high dose (6/50 vs 1/50 in control; p=0.051); and marginal increase incidence of carcinoma in the Zymbal's gland (3/50 vs 0/50 in control) in male rats (NTP 1992). At the lowest treatment dose 6000 ppm (corresponding to 215-275 mg/kg-bw/day in male), significant increase in incidence of squamous cell papillomas of the skin was also seen at the lowest dose (NTP 1992).</p>

Endpoint	Lowest effect levels ¹ /Results
	<p>Oral carcinogenicity in mice: Groups of 50 male and 50 female B6C3F1 mice were fed with diets containing 0 ppm, 12 500 ppm, 25 000 ppm, 50 000 ppm C.I. Pigment Red 3 for 2 years. In male mice, significant increases of renal tubule adenoma were observed in kidney in high-dose group (0/50, 0/50, 0/50, 6/50 for 0, 12 500, 25 000, 50 000 ppm, respectively; p=0.017, logistic regression trend test); and of follicular-cell adenoma in thyroid gland in the high-dose group (0/50, 0/49, 1/50, 5/50 for 0, 12 500, 25 000, 50 000 ppm, respectively; p=0.027, logistic regression trend test). No treatment-related tumours were seen in female mice (NTP 1992).</p> <p>Lowest oral LOAEL: 325 mg/kg-bw/day (6000 ppm in diet) was identified based on significantly increased incidence of liver lesions (eosinophilic focus, mixed cell focus, cystic degeneration and multifocal angiectasis); and increased severity of nephropathy and increased incidence of hyperplasia in kidney papilla transitional epithelium in male rats exposed to C.I. Pigment Red 3 in diet (0 ppm, 6000 ppm, 12 500 ppm, 25 000 ppm) for 2 years (NTP 1992).</p> <p>No inhalation or dermal studies identified.</p>
Developmental and reproductive toxicity	No experimental data available.
Genotoxicity and related endpoints: <i>in vivo</i>	<p>Comet assay: Significant increases of DNA damages measured by comet assay were observed in colon tissue in male ddY mice 24 hour after oral treatment with 2000 mg/kg-bw C.I. Pigment Red 3. No DNA damages were observed in other organs (Tsuda et al.2000).</p> <p>Micronuclei formation: No significantly increased induction of micronuclei in bone marrow cells and spleen in mice after injection into the peritoneum (Barański et al.1992)</p>
Genotoxicity and related endpoints: <i>in vitro</i>	<p>Mutagenicity: Positive: <i>Salmonella typhimurium</i> TA98, TA100 in the presence of induced hamster liver S9 (Mortelmans et al.1986; NTP 1992). Equivocal: <i>Salmonella typhimurium</i> TA98, TA100 in the presence of induced rat liver S9 (Mortelmans et al.1986; NTP 1992). Negative: <i>Salmonella typhimurium</i> TA98, TA100 in the absence of S9 (Mortelmans et al.1986; NTP 1992). Negative: <i>Salmonella typhimurium</i> TA1535, TA1537 with and without induced rat liver S9 activation (Mortelmans et al.1986; NTP 1992). Negative: <i>Salmonella typhimurium</i> TA98, TA100 in the presence of induced rat liver S9 activation (Miyagoshi et al.1983; Barański et al.1992, reviewed in Moller and Wallin 2000).</p> <p>Chromosome aberrations: Negative: Chinese hamster ovary (CHO) cells with and without of induced rat liver S9 activation (NTP 1992). Sister chromatid exchange: Negative: Chinese hamster ovary (CHO) cells with and without of induced rat liver S9 activation (NTP 1992).</p>
Humans	
Dermal toxicity	Slight erythema or erythema with edema were observed in patients with pigmented cosmetic dermatitis in patch or photopatch tests with C.I. Pigment Red 3 after application for 48 or 72 hours. Positive reactivity was 34.2% (13/38) (Sugai et al.1977). The reddening and swelling of the skin were seen in five of 155 patients with cosmetic

Endpoint	Lowest effect levels ¹ /Results
	dermatitis in a 48-h patch tests with 5% C.I. Pigment Red 3 in polyethylene glycol; slight reddening was seen in another 25 of the patients (MJCDRG 1978). In a patch test, negative reaction was observed with 1% Pigment Red 3 (in petrolatum) in the skin of patients who were suffered from dermatitis caused by Brilliant Lake Red R. No skin reactions were seen in 28 healthy young female volunteers in a 48 hour patch test with 1% C.I. Pigment Red 3 in petrolatum (Kgzuka et al.1980).

¹ LD₅₀ = median lethal dose; LOAEL = lowest-observed-adverse-effect level.