Screening Assessment for the Challenge

Benzoic acid, 2,3,4,5-tetrachloro-6-cyano-, methyl ester, reaction products with 4-[(4-aminophenyl)azo]-3-methylbenzenamine and sodium methoxide
(MATCB)

Chemical Abstracts Service Registry Number
106276-78-2

Environment Canada
Health Canada

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Synopsis

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the Ministers of the Environment and of Health have conducted a screening assessment on benzoic acid, 2,3,4,5-tetrachloro-6-cyano-, methyl ester, reaction products with 4-[(4-aminophenyl)azo]-3-methylbenzenamine and sodium methoxide (MATCB), Chemical Abstracts Service Registry Number 106276-78-2. 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 and inherent toxicity to non-human organisms and is believed to be in commerce in Canada.

The substance MATCB was not considered to be a high priority for assessment of potential risks to human health, based upon application of the simple exposure and hazard tools developed by Health Canada for categorization of substances on the Domestic Substances List. Therefore this assessment focuses principally on information relevant to the evaluation of ecological risks.

MATCB is an **U**nknown or **V**ariable Composition, **C**omplex Reaction Products, or **B**iological Materials (UVCB) and used in Canada primarily as a textile dye. The substance is not naturally produced in the environment. It is not reported to be manufactured in Canada; however, between 100 and 1000 kg of the substance were imported within dyed raw materials into Canada in 2006.

Based on reported use patterns and certain assumptions, most of MATCB is expected to end up in solid waste disposal sites (90%), and the residue proportion is estimated to be released to sewer water (10%). The substance is not expected to be soluble in water or to be volatile; instead, it is expected to partition to particles because of its hydrophobic nature. For these reasons, after release to water, the substance will likely end up mostly in sediments and, to a lesser extent, in agricultural soil that has been amended with biosolids. It is not expected to be significantly present in air, hence it is not expected to be subject to long-range atmospheric transport.

Based on prediction of its physical and chemical properties, MATCB is expected to degrade slowly under aerobic conditions in the environment (in water, sediment and soil). Due to lack of experimental data relating to the bioaccumulation potential, an experimental value adjustment (EVA) method and new data for an analogue of MATCB were used in the assessment. This resulted in the prediction that MATCB has a low potential for bioaccumulation in the environment. The substance therefore meets the persistence criteria but does not meet the bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations*. In addition, experimental toxicity data for chemical analogues suggest that MATCB has a low to moderate potential to cause acute harm to aquatic organisms.

For this screening assessment, a very conservative exposure scenario was selected representing consumer use-related releases to the aquatic environment. The scenario

simulated discharge of MATCB to the aquatic environment due to the washing of dyed clothing. The predicted environmental concentrations (PECs) in water were well below the predicted no-effect concentrations (PNECs) calculated for sensitive aquatic species. Therefore, it is concluded that the substance 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.

The potential for exposure of the general population in Canada to MATCB from environmental media is expected to be negligible. Exposure of the general population in Canada to MATCB from consumer products is expected to be low given the intended purpose of the product (dyes in synthetic textiles), taking into consideration potential for incidental exposures, such as mouthing by toddlers. Due to the lack of experimental data on MATCB, upper-bounding exposure estimates were derived based on available data on the migration of disperse dyes from synthetic textiles.

No empirical health effects data were identified for MATCB. The outputs of Quantitative Structure-Activity Relationship (QSAR) predictions for genotoxicity and carcinogenicity were mixed. Information from analogues and potential MATCB metabolites suggests a potential hazard for genotoxicity endpoints.

Although limited data may suggest a potential hazard associated with MATCB, exposure of the general population in Canada based on the use of the substance in textiles is expected to be low, therefore the risk to human health is considered to be low.

Based on the information available, it is concluded that MATCB does not meet any of the criteria set out in section 64 of the *Canadian Environmental Protection Act, 1999*.

Because this substance is listed on the *Domestic Substances List*, its import and manufacture in Canada are not subject to notification under subsection 81(1). Given the potential hazardous properties of this substance, there is concern that new activities that have not been identified or assessed could lead to this substance meeting the criteria set out in section 64 of the Act. Therefore, it is recommended to amend the *Domestic Substances List*, under subsection 87(3) of the Act, to indicate that subsection 81(3) of the Act applies with respect to the substance so that new manufacture, import or use of this substance is notified and undergoes ecological and human health risk assessments.

In addition and where relevant, research and monitoring will support verification of assumptions used during the screening assessment.

Introduction

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

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

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

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

The substance benzoic acid, 2,3,4,5-tetrachloro-6-cyano-, methyl ester, reaction products with 4-[(4-aminophenyl)azo]-3-methylbenzenamine and sodium methoxide (MATCB) 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 30, 2008 (Canada 2008). 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, a submission of information pertaining to the uses of the substance was received.

Although MATCB was determined to be a high priority for assessment with respect to the environment, it did not meet the criteria for GPE or IPE and high hazard to human health based on classifications by other national or international agencies for carcinogenicity, genotoxicity, developmental toxicity or reproductive toxicity (Canada 2006b).

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

scientific information and develop conclusions by incorporating a weight-of-evidence approach and precaution

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 April 2009 for ecological sections and December 2009 of human health sections of the document. Key studies were critically evaluated; modelling results may have been used to reach conclusions. When available and relevant, information presented in hazard assessments from other jurisdictions was considered.

The screening assessment does not represent an exhaustive or critical review of all available data. Rather, it presents the most critical studies and lines of evidence pertinent to the conclusion.

This screening assessment was prepared by staff in the Existing Substances Programs at Health Canada and Environment Canada and incorporates input from other programs within these departments. The ecological portion of this assessment has undergone external written science review/consultation. Additionally, the draft of this screening assessment was subject to a 60-day public comment period. While external comments were taken into consideration, the final content and outcome of the screening risk assessment remain the responsibility of Health Canada and Environment Canada.

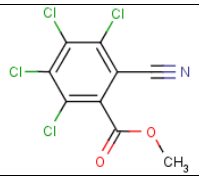
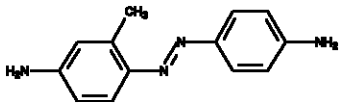
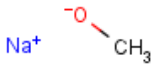
The critical information and considerations upon which the final assessment is based are summarized below.

Substance Identity

For the purposes of this document, this substance will be referred to as MATCB, derived from the Domestic Substances List (DSL) inventory name.

MATCB is an **U**nknown or **V**ariable Composition, **C**omplex Reaction Products, or **B**iological Materials (UVCB), which is not a discrete chemical and thus may be characterized by a variety of structures. In many databases, the substance is referred by the reaction chemicals of benzoic acid, 2,3,4,5-tetrachloro-6-cyano-, methyl ester, with 4-[(4-aminophenyl)azo]-3-methylbenzenamine and sodium methoxide. The chemical structures and categories of these three reaction chemicals are listed in Table 1.

Table 1. Three reaction chemicals for producing MATCB

Chemical names	CAS RN	Chemical Structure	Chemical categories
Benzoic acid, 2,3,4,5-tetrachloro-6-cyano-, methyl ester	5358-06-5		methyl ester
4-[(4-Aminophenyl)azo]-3-methylbenzenamine	43151-99-1		amine
Methanol, sodium salt (sodium methoxide)	124-41-4		catalyst

Manufacturing methods are well established for an amine reacting with an ester where the amine (the stronger base) and ester are consumed in the reaction, and an alcohol (a weaker base) and an amide are produced. Sodium methoxide is used as the catalyst in the reaction. For the chemical reaction of benzoic acid, 2,3,4,5-tetrachloro-6-cyano-, methyl ester, with 4-[2-(4-aminophenyl)diazenyl]-3-methylbenzenamine, there could be an amine-ester reaction on one end of the amine (CAS RN 43151-99-1), or on both ends.

As indicated by the Toxic Substances Control Act (TSCA) Chemical Substance Inventory of the United States Environmental Protection Agency (US EPA), MATCB is generated from a reaction between the amine and the ester controlled as 1:1; therefore, it is believed that MATCB is intentionally assigned to represent the substance from the single amine-ester reaction between CAS RN 5358-06-5 (chemical 1) and CAS 43151-99-1 (chemical 3) and, as presented in Figure 1 below.

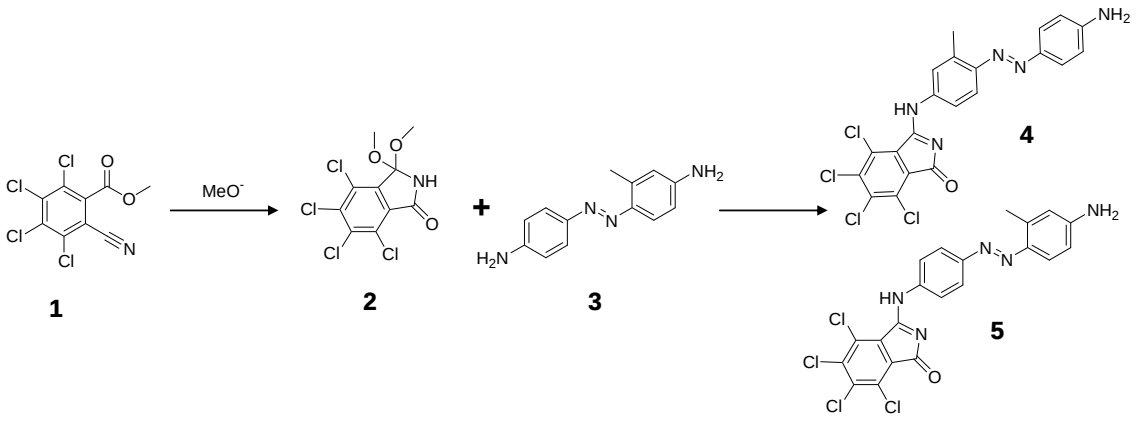


Figure 1. The single-amine-ester-reaction which produces two isomers of MATCB (CAS RN 106276-78-2)

There are two isomers of MATCB from the single amine-ester reaction, chemical 4 and chemical 5, as illustrated in Figure 1. Of the two amines (-NH_2) in 4-[(4-Aminophenyl)azo]-3-methylbenzenamine (CAS 43151-99-1), the one on the methyl substituted ring is slightly more basic; thus, the reaction is more likely to occur at this site and produce a higher percentage of chemical 4 than chemical 5. Therefore, this isomer (chemical 4) will be used as the representative structure of MATCB (CAS RN 106276-78-2) in the assessment (Table 3). Meanwhile, it is anticipated that there is no significant difference between these two isomers in terms of their physical and chemical properties and toxicity.

With a sufficient amount of ester (chemical 1), the amine-ester reaction would take place on both ends of the amine and then produce Pigment Orange 61 (CAS 40716-47-0; chemical 6 in Figure 2) in the highest proportion. Pigment Orange 61 is another discrete compound from the amine-ester reactions as presented in Figure 2 below.

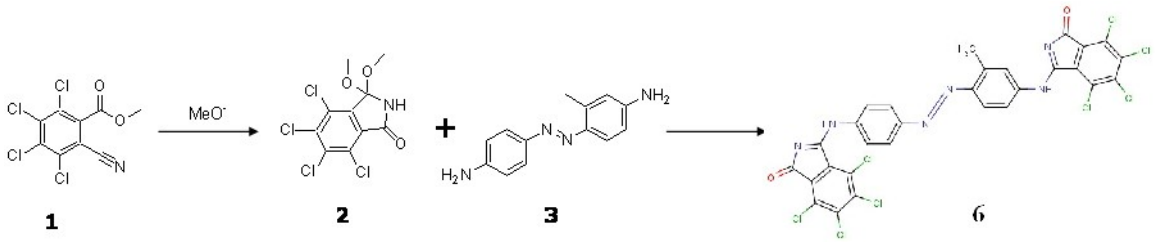
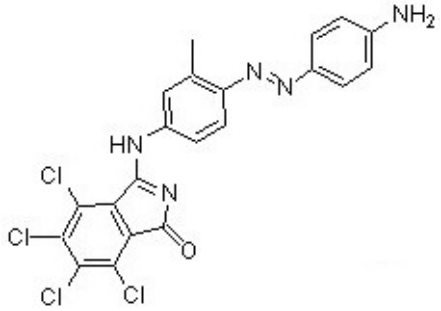


Figure 2. The double-amine-ester-reaction which produces Pigment Orange 61 (CAS RN 40716-47-0)

Under either of the above conditions (as illustrated in Figures 1 and 2), the final products could be a mixture, containing both of MATCB and Pigment Orange 61, as well as residuals of the reactants. The substances of interest may be present in higher or lower concentration in the mixture, depending on the control of reaction conditions.

Given the lack of empirical data for MATCB, experimental data on the aquatic toxicity of Pigment Orange 61 are used in the assessment.

Table 2. Substance identity for MATCB

Chemical Abstracts Service Registry Number (CAS RN)	**106276-78-2**
DSL name	**Benzoic acid, 2,3,4,5-tetrachloro-6-cyano-, methyl ester, reaction products with 4-[(4-aminophenyl)azo]-3-methylbenzenamine and sodium methoxide**
National Chemical Inventories (NCI) names[1]	*Benzoic acid, 2,3,4,5-tetrachloro-6-cyano-, methyl ester, reaction products with 4-[2-(4-aminophenyl)diazenyl]-3-methylbenzenamine and methanol sodium salt (1:1)* (TSCA) *Benzoic acid, 2,3,4,5-tetrachloro-6-cyano-, methyl ester, reaction products with 4-[(4-aminophenyl)azo]-3-methylbenzenamine and sodium methoxide* (AICS, ASIA-PAC)
Other names	*Reaction product of benzoic acid, 2,3,4,5-tetrachloro-6-cyano-, methyl ester with sodium methylate and benzeneamine, 4-[(4-amino-phenyl)azo]-3-methyl-*
Chemical group (DSL Stream)	UVCB[2]
Major chemical class or use	UVCB – organic
Major chemical sub-class	UVCB - organic disperse azo dye
Chemical formula	$C_{13}H_{14}N_4.C_9H_3Cl_4NO_2.CH_4O.Na$
Representative chemical structure used to run the estimation model[2]	
Representative SMILES[3] used to run the estimation model	c1cc(N)ccc1N=Nc2c(C)cc(cc2)NC3=NC(=O)c4c(Cl)c(Cl)c(Cl)c(Cl)c34
Molecular mass	493.18 g/mol

[1] National Chemical Inventories (NCI). 2006: AICS (Australian Inventory of Chemical Substances); ASIA-PAC (Asia-Pacific Substances Lists); and TSCA (Toxic Substances Control Act Chemical Substance Inventory)

[2] This substance is a UVCB (**U**nknown or **V**ariable Composition, **C**omplex Reaction Products, or **B**iological Materials); i.e., it is not a discrete chemical and thus may be characterized by a variety of structures. To assist with modelling, the representative chemical structure and the corresponding SMILES have to be selected

[3] Simplified Molecular Input Line Entry System

Identification of Analogue Substances and Estimation of Physical and Chemical Properties

Few experimental data are available for MATCB.

At the Environment Canada-sponsored Quantitative Structure-Activity Relationship (QSAR) Workshop in 1999 (Environment Canada 2000), Environment Canada and other invited modelling experts identified many structural classes of pigments and dyes as "difficult to model" using QSARs. The physical and chemical properties of many of the structural classes of dyes and pigments (including acid and disperse dyes) are not amenable to model prediction because they are considered "outside the model domain of applicability" (e.g., structural and/or property parameter domains). Therefore, to determine the domain of applicability, Environment Canada reviews the applicability of QSAR models to dyes and pigments on a case-by-case basis.

It is considered inappropriate to use QSAR models to predict most of the physical and chemical properties of MATCB. Consequently, analogues were identified and "read-across" data were used to determine the approximate physical and chemical properties for the substance. These properties were summarized in Table 4 and subsequently used for furthering modeling and lines of evidence.

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

To find acceptable analogues, a review of data for several disperse azo dyes was performed (Anliker et al. 1981; Anliker and Moser 1987; Baughman and Perenich 1988; ETAD 2005; Brown 1992; Hine and Mookerjee 1975; Sijm et al. 1999; Safepharm Laboratories Ltd 1990; Sandoz 1975). These compounds are structurally similar to MATCB but also share other important attributes that contribute to their fate in the environment, such as high molecular weights—generally > 400 g/mol, similar cross-sectional diameters (1.35–1.90 nm), solid particulate structures, decomposition at greater than 120ºC (to 270ºC), and "dispersibility" in water (i.e., they are not truly soluble). In addition, they exert negligible vapour pressure and are stable under environmental conditions, as they are designed to be so.

In the case of MATCB, the unique chemical structure of the substance makes it difficult to find analogues that are both structurally close to MATCB and can provide experimental data to support the assessment. Therefore, azo dye substances with limited structural similarity to MATCB were also used to characterize physical and chemical parameters in a read-across approach (see Table 4). The structural differences between these azo dye substances and MATCB have been noticed, so that using read-across data is considered to be conservative in the assessment on MATCB.

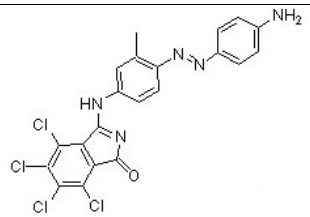
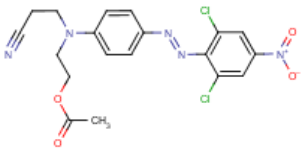
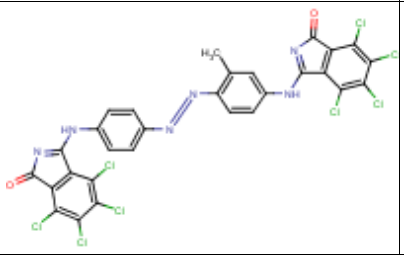
When collecting the physical and chemical properties and toxicity of MATCB, data for Pigment Orange 61 and Disperse Orange 30 have been given particular weight in the assessment. The notable differences between these substances and MATCB have been taken into account when data for MATCB was read-across from Pigment Orange 61 and Disperse Orange 30.

Pigment Orange 61 (CAS RN 40716-47-0) has been identified as an alternative to assess the aquatic toxicity of MATCB. Both chemicals contain similar functional groups. However Pigment Orange 61 has a higher molecular weight (760.08 g/mol) and a larger cross-sectional diameter (2.22–2.98 nm) compared to MATCB (Table 5); therefore it is expected that Pigment Orange 61 could be less bioavailable and consequently less toxic.

Disperse Orange 30 (CAS RN 5261-31-4) is another monoazo compound. Disperse Orange 30 and MATCB have similar molecular weights (450.28 g/mol and 493.18 g/mol, respectively). It should be noted that MATCB has a different cross-sectional diameter (1.29–2.20 nm) than Disperse Orange 30 (1.75–1.98 nm), and different functional groups (Table 5). However, such differences are not anticipated to cause significantly different environmental behaviours and toxicities. Also due to the structural difference, MATCB is expected to have a somewhat lower octanol-water partition coefficient than Disperse Orange 30. Therefore, it is considered that using read-across data from Disperse Orange 30 may be conservative and an appropriate response to the uncertainties associated with the assessment of bioaccumulation and ecological toxicity for MATCB.

Some properties of Pigment Orange 61 and Disperse Orange 30, as well as the types of experimental data available, are summarized in Table 3 below and subsequently used for furthering modeling and lines of evidence in the assessment

Table 3. Azo compounds used to support the assessment of MATCB

CAS RN (Common Name)	Chemical Structure	Molecular Mass (g/mol)	Min-Max Cross-Sectional Diameter (nm)[1]	Available Empirical Data
106276-78-2 (MATCB)		493.18	1.29–2.20	
5261-31-4 (Disperse Orange 30)		450.28	1.75–1.98	Melting point, vapour pressure, log K_{ow}, water solubility, aquatic toxicity
40716-47-0 (Pigment Orange 61)		760.08	2.22–2.91	Aquatic toxicity

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

Physical and chemical properties of MATCB and 'read-across' of other dyes are summarized in Table 4 below.

Table 4. Physical and chemical properties of MATCB and other azo dyes

Chemicals	Type[1]	Value	Temperature (°C)	Reference
Melting point (ºC)[2]				
Disperse Orange 30	Experimental	126.9–128.5		ETAD 2005
Read-across for azo dyes	Experimental	117–225		Anliker and Moser 1987
		74–236		Baughman and Perenich 1988
Boiling point (ºC)[3]				
Not applicable				
Vapour pressure (Pa)				
Read-across for azo dyes	Experimental	5.33×10^{-12} to 5.33×10^{-5} (4×10^{-14} to 4×10^{-7} mm Hg)		Brown 1992
Henry's Law constant ($Pa\cdot m^3/mol$)				
Read-across for azo dyes[4]	Experimental	> 2–5.1		Baughman and Perenich 1988
Log K_{ow} (octanol-water partition coefficient) (dimensionless)				
Disperse Orange 30	Experimental	4.2		Brown 1992
Read-across for azo dyes	Experimental	1.79–5.07		Baughman and Perenich 1988
Read-across for azo disperse dyes	Experimental	> 2–5.1		Anliker et al. 1981; Anliker and Moser 1987
Log K_{oc} (organic carbon-water partition coefficient) (dimensionless)				
Read-across for azo dyes	Calculated[5]	3.4 – 4.2		Baughman and Perenich 1988
Water solubility (mg/L)				
Disperse Orange 30	Experimental	0.07		Brown 1992
Read-across for azo disperse dyes	Experimental	< 0.01		Anliker and Moser 1987
Read-across for azo dyes	Experimental	$1.2\times\times10^{-5}$ to 35.5 (4×10^{-11} to 1.8×10^{-4} mol/L)		Baughman and Perenich 1988
n-octanol solubility (mg/L)				
Disperse Orange 30	Experimental	576		ETAD 2005
Read-across for azo disperse dyes	Experimental	81–2430	20	Anliker and Moser 1987
pK_a (acid dissociation constant) (dimensionless)				
MATCB	Modelled	9.77 (acid form) 3.09 (base form)		ACD/pK_aDB 2005

[1] The extrapolated values used for MATCB are based on available experimental evidence from other dye analogues found in the literature.
[2] The phrase "melting point" is used but this may be better described as a decomposition point because dyes are known to char at high temperatures (greater than 200°C) rather than melt.
[3] Boiling point is generally not applicable for dyes. For powder dyes, charring or decomposition occurs at high temperatures instead of boiling. For liquids and pastes, boiling will only occur for the solvent component, while the unevaporated solid will decompose or char (ETAD 1995).
[4] Solubilities of azo dyes at 25°C and 80°C were used by Baughman and Perenich (1988) to calculate Henry's Law constants for these dyes. These values are presented here as a range to illustrate the expected Henry's Law constant for MATCB.
[5] Log K_{oc} values are based on calculations by Baughman and Perenich (1988) using a range of measured solubilities for commercial dyes and an assumed melting point of 200°C.

Sources

MATCB is not reported to be naturally produced in the environment.

The quantity reported to the Domestic Substances List (DSL) as being manufactured, imported or in commerce in Canada during the 1986 calendar year was 100–1000 kg.

Recent information was collected through an industry survey conducted for the 2005 and 2006 calendar years under the *Canada Gazette* notices issued pursuant to section 71 of CEPA 1999 (Canada 2006b and Canada 2008). These notices required submission of data on the Canadian manufacture and import of MATCB. In the notice for 2006, data were also required on the use quantity of this substance.

No manufacture or import of MATCB was reported above the 100 kg/year threshold in the 2005 calendar year.

In the 2006 calendar year, no manufacture of MATCB was reported above the 100 kg/year threshold. However, 100–1000 kg of the substance was imported into Canada within dyed raw materials. Using the Declaration of Stakeholder Interest form associated with the section 71 survey for 2006, four companies reported a stakeholder interest for this substance.

Uses

Information on uses for the 2005 and 2006 calendar years was gathered in response to the CEPA 1999 section 71 notices (Canada 2006b and 2008). Some uses of MATCB have not been identified in this document, as this information has been requested to be treated as confidential business information. However, these uses have been considered in evaluation of potential risk of the substance.

The following DSL use code was identified for MATCB during the DSL nomination period (1984–1986): 13 – Colourant - Pigment, Stain, Dye and Ink (Environment Canada 1988).

Use of this substance other than as a colorant for textile fibre has not been identified.

Releases to the Environment

Mass Flow

To estimate potential releases of substances to the environment at different stages of their life cycle, a Mass Flow Tool was developed (Environment Canada 2008a). 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 is 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 off-site releases to the environment from waste disposal sites.

Assumptions and input parameters used in making the release estimates are based on information obtained from a variety of sources, including responses to regulatory surveys, Statistics Canada, manufacturers' websites, technical databases and documents, and professional knowledge and assumptions. 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 increases toward the end of the life cycle.

Table 5. Estimated releases and losses of MATCB to environmental media, chemical transformation during life cycle and transfer to waste disposal sites, based on the Mass Flow Tool

Fate		Proportion of the mass (%)[1]	Major life cycle stage involved[2]
Released to receiving media:			
	To soil	0.0	n/a[3]
	To air	0.0	n/a
	To wastewater[3]	10.0	Consumer use
Chemically transformed (incineration)		2.7	Waste disposal
Transferred to waste disposal sites (e.g., landfill)		87.3	Waste disposal

[1] For MATCB, information from the following OECD emission scenario documents was used to estimate releases to the environment and the distribution of the substance as summarized in this table: Textile Manufacturing Wool Mills (OECD 2004), and Adhesive formulation (OECD 2007). Specific assumptions used in the derivation of these estimates are summarized in Environment Canada 2008b.

[2] Applicable stage(s): production, formulation, industrial use, consumer use, service life of article/product, waste disposal.
[3] Wastewater before any form of treatment, either on-site industrial or off-site municipal wastewater treatment.

Based on Statistics Canada information and an analysis by Industry Canada (2008), it is recognized that dyes may be imported in manufactured articles. For estimating the risk associated with potential releases and losses of MATCB to environmental media, the ratio of textiles manufactured in Canada to imported textiles of 30:70 has been used to estimate the amount of dye imported in finished textiles (Industry Canada 2008; Environment Canada 2008b). This import quantity was included in the Mass Flow Tool calculations as well as in the exposure scenarios developed further.

Results from the Mass Flow Tool indicate that MATCB can be expected to be found largely in waste disposal sites (90%), due to the eventual disposal of manufactured items that contain MATCB. A small fraction (2.7%) of solid waste is incinerated, which is expected to result in chemical transformation of the substance. Based largely on information contained in OECD emission scenario documents for uses associated with this type of substance (OECD 2004, 2007), it is estimated that 10.0% of MATCB may be released to wastewater, mainly resulting from activities associated with the consumer use of products containing the substance. Although not considered in the Mass Flow Tool, the substance may be applied to agricultural soils and pasture lands in Canada as a component of biosolids that are commonly used for soil enrichment.

Although a significant fraction of the substance will find its way into landfill sites through the disposal of manufactured items, the sewer water is considered the critical medium for MATCB due to consumer use. Therefore, the potential for direct exposure of biota in the aquatic environment is of the major focus in the assessment.

Environmental Fate

As indicated by the results of the Mass Flow Tool (Table 6), MATCB is expected to be released to wastewater mostly during consumer use. The moderate to high log K_{ow} value (read-across of 1.8 to 5.1) and high log K_{oc} (read-across of 3.4 to 4.2) values (see Table 4) indicate that this dye may have affinity for solids. However, the log K_{oc} is a calculated value (see footnote 5 below Table 4), and the adsorption potential of disperse particulate dye structures is generally not well understood; therefore, the degree to which this particular behaviour applies to MATCB is uncertain.

According to aerobic biodegradation models, MATCB is not expected to biodegrade quickly (see Table 7 below).

Given its estimated pKa values (9.77 acid, 3.09 base), it is unlikely that ionization will have a significant impact on the partitioning or water solubility of the substance. Because

of its low solubility, when released into water it is expected to behave as a colloidal dispersion (Yen et al. 1991). It will therefore mostly be present as solids or adsorbed to suspended particles and will eventually sink to bed sediments. Razo-Flores et al. (1997) have stated that, due to the recalcitrant nature of azo dyes in the aerobic environment, they eventually end up in anaerobic sediments due to sediment burial, or in shallow aquifers (groundwater).Yen et al. (1991) observed that an azo benzothiazole dye was transformed under anaerobic conditions in sediment via hydrolysis and reduction, and concluded that most azo dyes would likely not persist in anaerobic sediment systems.

The rate of volatilization from the surface of water is proportional to the Henry's Law constant (Baughman and Perenich 1988). Baughman and Perenich (1988) also state that volatilization from aquatic systems will not be an important loss process for dyes, which agrees with the low to negligible read-across Henry's Law constant value (10^{-8} to 10^{-1} Pa•m^3/mol, Table 5). Transfer to air due to the loss of these substances from moist and dry soil surfaces is not likely to be significant for these substances, as indicated by very low read-across vapour pressures for disperse azo dyes (5.33×10^{-12} to 5.33×10^{-5} Pa) (Table 5). These data are consistent with the physical state (solid particle) of MATCB, which makes it an unlikely candidate for volatilization.

Persistence and Bioaccumulation Potential

Environmental Persistence

No experimental degradation data for MATCB have been identified.

According to the Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers, dyes, with some exceptions, are considered essentially non-biodegradable under aerobic conditions (ETAD 1995). Repeated evaluation of ready and inherent biodegradability using accepted screening tests (see OECD Guidelines for Testing Chemicals) have confirmed this for such chemicals (Pagga and Brown 1986; ETAD 1992). Based on the representative chemical structure of MATCB, there is no reason to suspect that biodegradation will be other than that of dyes generally (ETAD 1995).

Disperse dyes enter the aquatic system mostly as a dispersion of fine suspended particles, eventually settling to the aerobic layers of surface sediment where they will persist until sediment burial creates reducing conditions. The rate of sediment deposition and the extent of bioturbation vary from site to site, and thus it is very difficult to ascertain the residence time of dyes in aerobic sediment layers. However, it is likely that, in many cases, this is greater than 365 days. Once under anaerobic or reducing conditions, azo dyes may undergo rapid degradation to substituted aromatic amine constituents as demonstrated by Yen et al.(1989), who measured reduction half-life values in compacted sediments at room temperature of 1.9–2.0 days for an azo benzothiazole dye (CAS RN 68133-69-7). However, in deep anoxic sediment, these biodegradation transformation products are not expected to present a high degree of exposure potential to most aquatic

organisms. This is in part because contact of organisms with anoxic sediment is likely to be limited, and also because the amine degradation products are expected to be irreversibly bound to sediments, resulting in very low bioavailability (Weber et al. 2001; Colon et al. 2002). Therefore, they are not likely to present an ecological concern.

Given the expected release of MATCB into wastewater, persistence was primarily examined using predictive QSAR biodegradation models, which are considered acceptable for use in this situation as these models are based on chemical structure and the azo structure is represented in the training sets of all the degradation models used, thereby increasing the reliability of these predictions. The following analysis applies primarily to the portion of a substance that is present in the environment in the dissolved form, recognizing that the largest proportion would likely exist in dispersed form as solid particles. MATCB does not contain functional groups expected to undergo hydrolysis in aerobic environments, as dyes are designed to be stable in aqueous conditions.

Table 6 summarizes the results of available QSAR models for the degradation of MATCB in water.

Table 6. Modelled data for degradation of MATCB in water

Fate Process	Model and Model Basis	Model Output	Expected Half-life (days)
Biodegradation (aerobic)	BIOWIN 2000 Sub-model 3: Expert Survey (ultimate biodegradation)	0.6378[1] (biodegrades very slowly)	> 182
Biodegradation (aerobic)	BIOWIN 2000 Sub-model 5: MITI linear probability	-0.9927[2] (biodegrades very slowly)	> 182
Biodegradation (aerobic)	BIOWIN 2000 Sub-model 6: MITI non-linear probability	0.0[2] (biodegrades very slowly)	> 182
Biodegradation (aerobic)	CATABOL c2004–2008 % BOD (biological oxygen demand)	0.013 (biodegrades very slowly)	> 182

[1] Output is a numerical score.
[2] Output is a probability score.

The results from Table 7 indicate that all aerobic biodegradation models (BIOWIN 3, 5, 6 and CATABOL) suggest that MATCB biodegrades slowly. In fact, both of the BIOWIN 5 and 6 probability results are much less than 0.3, the cut-off value suggested by Aronson et al. (2006) to identify a substance as having a half-life > 60 days (based on the MITI probability models). Furthermore, both of the other ultimate degradation models, BIOWIN 3 and CATABOL, predict that this substance will be persistent in water.

When the results of the probability and the other degradation models are considered, there is model consensus that the ultimate biodegradation half-life in water is > 182 days.

This finding is consistent with what would be expected for this chemical's structure (i.e., few degradable functional groups).

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

Based on the modelled data (see Table 7 above), MATCB meets the persistence criteria in water, soil and sediment (half-lives in soil and water ≥ 182 days and half-life in sediment ≥ 365 days), as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

As noted previously, MATCB is not expected to be volatile or significantly present in air. Hence it is not expected to be subject to long-range atmospheric transport.

Potential for Bioaccumulation

No experimental bioaccumulation experimental data are available for MATCB.

For many non-soluble dye classes, including non-soluble azo dyes, it is difficult to model the bioaccumulation potentials, and thus the results are generally unreliable. Predicted and/or empirically determined properties of dyes related to bioaccumulation (e.g., log K_{ow}) can be of uncertain relevance or associated with a high degree of error, which reduces the utility of model predictions of BCF and BAF. In addition, monoazo dyes generally fall outside of bioaccumulation model domains of applicability. As a result, in this assessment, bioaccumulation modelling has not been used to evaluate the bioaccumulation status of MATCB.

In the absence of experimental and modelled data specific to MATCB, a bioconcentration factor (BCF) estimated for Disperse Orange 30 was used to provide an indication of bioaccumulation potential for MATCB. It is noted that Disperse Orange 30 and MATCB have similar molecular weights (450.28 g/mol and 493.18 g/mol, respectively), but different cross-sectional diameters (MATCB: 1.29–2.20 nm and Disperse Orange 30: 1.75–1.98 nm) and different functional groups (see Table 5). It is anticipated that MATCB may demonstrate a lower octanol-water partition coefficient than Disperse Orange 30 based on the model predictions. Utilizing read-across data from a chemical with a higher octanol-water partition coefficient is conservative because this characteristic can be associated with bioaccumulative substances.

A bioconcentration study submitted for Disperse Orange 30 suggests that it is unlikely to accumulate in fish (Shen and Hu 2008). This test was performed according to the OECD Guidelines for Testing of Chemicals, Test No. 305B, Bioconcentration: Semi-Static Fish Test (OECD 1996). The bioconcentration of Disperse Orange 30 in zebra fish (*Brachydanio rerio*) was determined in a 28-day semi-static test with a test medium

renewal every two days. A nominal concentration of 20 mg/L (mean measured concentration 0.028 ~ 0.28 mg/L) was used in study to check the bioconcentration potential of the test substance. Samples from both test solutions and test organisms were taken daily from Day 26 to Day 28 of the 28-day exposure test period. Samples were prepared by extracting the lipid component from the test fish. The measured concentrations of test substance, fish lipid contents and BCF calculations are summarized in Table 7 below.

Table 7. Measured concentrations of Disperse Orange 30, fish lipid contents and BCF calculations

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

The Shen and Hu (2008) study was reviewed and considered acceptable (see Appendix 3). Lack of detection of Disperse Orange 30 in fish extracts (< 0.028 mg/L) suggests a limited solubility in lipids and/or limited potential to partition into fish tissue from aqueous systems. Assuming that the concentration in solution in the test was equal to the lowest water solubility value of 0.028 mg/L, and using the fish concentration of 0.81 mg/kg as a worst-case estimate, the BCF may be calculated to be < 100. However, there is some uncertainty associated with limit-bounded values in any study because the "true" value is not known. But given the structure and likely behavior of MATCB and the analogue in aqueous systems, the low BCF result is expected.

Most disperse dyes exist as fine dispersible particles with limited truly soluble fractions. Solubility, however, can be increased by adding functional groups to the molecule. Disperse Orange 30 contains some of these solubilizing groups (nitroso); thus some degree of water solubility would be expected. MATCB does not contain any functional groups expected to be ionic at relevant environmental pHs of 6–9.

While the above study serves as primary evidence to support the expectation that MATCB lacks bioaccumulation potential, other research corroborates this conclusion. Anliker et al. (1981) reported experimental fish bioaccumulation values for 18 disperse monoazo dyes, performed according to test methods specified by the Japanese Ministry

of International Trade and Industry (MITI). Expressed on the basis of wet body weight of the fish, these log bioaccumulation factors ranged from 0.00 to 1.76 (Anliker et al. 1981). Chemical registry numbers and chemical structures were not reported in this study and therefore limited the utility of this study for read-across purposes to MATCB. However, follow-up studies, which provided the chemical structures for the disperse dyes tested, confirmed low bioaccumulation potential for 10 nitroazo dyes, with reported log bioaccumulation factors ranging from 0.3 to 1.76 (Anliker and Moser 1987; Anliker et al. 1988). Studies available from MITI also support low bioaccumulation potential for disperse azo dyes. Reported BCFs for 3 disperse azo dyes (CAS RNs 40690-89-9, 61968-52-3 and 71767-67-4) tested at a concentration of 0.01 mg/L were in the range of < 0.3 to 47 (MITI 1992). An accumulation study by Brown (1987) also showed that none of the 12 disperse dyes tested accumulated during an 8-week study with carp.

Moderate to high log K_{ow} values for other disperse azo dyes, including Disperse Orange 30 (Table 4), is the only line of evidence suggesting that MATCB may have a high potential for bioaccumulation. In spite of the high log K_{ow} values for Disperse Orange 30 and the other azo substances, evidence for bioaccumulation of disperse azo dyes is lacking (Anliker et al. 1981; Anliker and Moser 1987; Anliker et al. 1988; MITI 1992). Authors who have measured high log K_{ow}s and concomitant low bioaccumulation factors for disperse azo dyes suggest that the low accumulation factors may be due to their low absolute fat solubility (Brown 1987) or relatively high molecular weight, which may make transport across fish membranes difficult (Anliker et al. 1981; Anliker and Moser 1987). It is also likely that the lack of bioavailability and limited capacity to partition under BCF test conditions limits accumulation in fish lipids.

It has been stated by ETAD (1995) that the molecular characteristics indicating the absence of bioaccumulation are a molecular weight of > 450 g/mol and a cross-sectional diameter of > 1.05 nm. Recent investigation by Dimitrov et al. (2002), Dimitrov et al. (2005) and the BBM (2008) suggests that the probability of a molecule crossing cell membranes as a result of passive diffusion declines significantly with increasing maximum cross-sectional diameter (D_{max}). Sakuratani et al. (2008) have also investigated the effect of cross-sectional diameter on passive diffusion in a test set of about 1200 new and existing chemicals. They observed that substances that do not have a very high bioconcentration potential often have a D_{max} of > 2.0 nm and an effective diameter (D_{eff}) of > 1.1 nm.

MATCB has a molecular weight of 493.18 g/mol and a D_{max} = 2.2 nm, indicating a potential for a significantly reduced uptake rate from water and reduced in vivo bioavailability of the substance.

Based on a lack of observed accumulation in bioconcentration tests with similar azo substances and the molecular size of MATCB, the substance is considered to have a low potential for bioaccumulation. It is therefore concluded that MATCB does not meet the bioaccumulation criteria (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 are no experimental data available for aquatic toxicity for MATCB. Data for Pigment Orange 61 and Disperse Orange 30 were therefore used as the closest analogue data to fill the data gap for evaluating the potential of MATCB to cause ecological effects.

Both chemicals of Pigment Orange 61 and MATCB contain the same functional groups in their molecules, however Pigment Orange 61 has a higher molecular weight (760.08 g/mol) and a larger cross-sectional diameter (2.22–2.98 nm) compared to MATCB (Table 5). It is expected that Pigment Orange 61 could be less bioavailable and consequently less toxic. However, as discussed in the fate and bioaccumulation sections, the bioavailability of MATCB is also expected to be low.

According to the Material Safety Data Sheet (MSDS) published by Kremer Pigmente (Kremer 2003), Pigment Orange 61 has an LC_{50} > 100 mg/L for carp (Table 9). However, the experimental details of this study were not provided, which does not allow evaluation of the study. In the same MSDS, a value for Pigment Orange 61 for inhibition of growth of wastewater bacteria of IC_{50} > 100 mg/L was reported. The study was conducted under the OECD 209 guideline (Kremer 2003) without, however, fully specifying the test conditions.

The empirical toxicity data for Disperse Orange 30 were also taken into consideration (Table 9). The structural difference between Disperse Orange 30 and MATCB has been noticed. MATCB is expected to have a somewhat lower octanol-water partition coefficient, so it is considered that using read-across data from Disperse Orange 30 may be conservative and an appropriate response to the uncertainties associated with the assessment of ecological toxicity for MATCB.

A study submitted on behalf of ETAD provides acute ecotoxicity data for fish, invertebrates, algae and bacteria for Disperse Orange 30 (Brown 1992). A 96-hr LC_{50} of 710 mg/L for zebra fish, a 48-hour EC_{50} of 5.8 mg/L for *Daphnia magna,* and a 72-hour EC_{50} (for growth) of 6.7 mg/L for *Scenedesmus subspicatus* have been reported based on toxicity studies using Disperse Orange 30 (Table 9); however, the original studies have not been provided to allow verification of their reliability.

Another result for Disperse Orange 30 was submitted to Environment Canada as a voluntary data submission. An LC_{50} for rainbow trout (*Oncorhynchus mykiss*) was established to be > 700mg/L (Sandoz 1975). An evaluation was conducted based on the robust study summary and it was concluded that the study (Sandoz 1975) was unacceptable (see Appendix 3).

An acute toxicity study with Disperse Orange 30 using rainbow trout (LC_{50} reported as > 100 mg/L) was also submitted to Environment Canada (Table 9) (Safepharm Laboratories Ltd. 1990). An assessment of the reliability of the study using a robust study summary was conducted, and the study was deemed to be of low confidence due to lack of details (Appendix 3).

Table 8. Empirical data for aquatic toxicity of Pigment Orange 61 and Disperse Orange 30

Test Chemicals and CAS RN	Test Organism	Type of Test and Duration (hours)	End Point	Value (mg/L)	Reliability of the Study	Reference
Pigment Orange 61 (40716-47-0)	Carp	Acute 96	LC_{50}[1]	> 100	Not available	Kremer 2003
	Bacteria	Acute 96	IC_{50}[2]	> 100	Acceptable	
Disperse Orange 30 (5261-31-4)	Rainbow trout (*Oncorhynchus mykiss)*	Acute 48	LC_{50}	> 700	Unacceptable	Sandoz 1975
	Rainbow trout *(Salmo gairdneri)*	Acute 96	LC_{50}	> 100	Low confidence	Safepharm Laboratories Ltd 1990
	Zebra fish	Acute 96	LC_{50}	710	Not available	Brown 1992
	Daphnia magna	Acute 48	EC_{50}[3]	5.8		
	Scenedesmus subspicatus	Acute 72	EC_{50}	6.7		
	Bacteria	Acute	IC_{50}	> 100		

[1] LC_{50} − The concentration of a substance that is estimated to be lethal to 50% of the test organisms.
[2] IC_{50} − The concentration of a substance that is estimated inhibit growth in 50% of the test organisms.
[3] EC_{50} − The concentration of a substance that is estimated to have some toxic sublethal effect on 50% of the test organisms.

Results of toxicity studies with Pigment Orange 61 and Disperse Orange 30 are generally consistent with those of a number of other toxicity studies with azo dyes, which report acute effect values (LC_{50} and EC_{50}s) in the range of 7 to 505 mg/L for fish, invertebrates and algae (Environment Canada 1995; Brown 1992; Cohle and Mihalik 1991; Little and Lamb 1973).

In general, due to their very low water solubility (< 1 mg/L), disperse dyes are expected to have a low acute ecological impact (Hunger 2003). The results of empirical toxicity studies with both Disperse Orange 30 and several similar azo dyes are consistent with this expectation, with *Daphnia* generally being the most sensitive organisms tested. Although interpretation of results from these tests is complicated by the fact that the reported effect values (i.e., EC_{50}s and LC_{50}s) are likely to be much greater than the solubility of the

substances tested and are likely the result of indirect toxic effects, the data available do indicate that the toxicity of MATCB is likely to be low.

A range of aquatic toxicity predictions for MATCB and analogues were also obtained from QSAR models. However, as with bioaccumulation, these QSAR ecotoxicity predictions for these dyes are not considered reliable because of the potential error associated with input parameters and the unique nature of disperse dyes, as well as structural and/or physical and chemical properties that fall outside of the models' domain of applicability.

The available empirical ecotoxicity information for other azo dye compounds indicates that MATCB is not likely to be highly hazardous to aquatic organisms.

B - In Other Environmental Compartments

Since MATCB is expected to accumulate in sediment and may potentially enter soil from biosolids that are commonly used for soil enrichment, as well as from the disposal of products that degrade and release the substance, it would be desirable to have toxicity data for sediment and soil organisms. However, no suitable ecological effects studies were found for MATCB or its analogues in media other than water. Although no suitable ecological effects studies were found for this compound in soil, considering the analogue toxicity data for aquatic organisms as well as the lack of bioaccumulation potential and its low bioavailability, potential for toxicity to soil-dwelling organisms is likely to be low. For the same reasons, the toxicity potential is also likely to be low in sediment-dwelling species, although this cannot be substantiated due to the lack of suitable whole-organism toxicity data. In addition, the toxicity potential of MATCB in anoxic sediments will likely be low because of the low bioavailability of their anaerobic degradation products.

Ecological Exposure Assessment

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

As this substance is found primarily in consumer products in Canada, Mega Flush, Environment Canada's spreadsheet model for estimating down-the-drain releases from consumer uses, was used to estimate the potential substance concentration in multiple water bodies receiving sewage treatment plant effluents to which the substance may have been released (Environment Canada 2008c). The spreadsheet model is designed to provide these estimates based on conservative assumptions regarding the amount of substance(s) used and released by consumers.

The total quantity of MATCB used by consumers was estimated by taking into consideration the ratio of 30:70 for textiles manufactured in Canada versus textiles imported. The following assumptions were made: the fraction lost to sewers associated with consumer use was 10%; wastewaters received only primary treatment with a 60% removal efficiency; and flow conditions were low (10th percentile values) in the receiving water bodies. The overall effect of these assumptions was to make this scenario moderately conservative.

This resulted in a maximum predicted environmental concentration (PEC) of 5.4×10^{-5} mg/L (Environment Canada 2008d).

Characterization of Ecological Risk

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

Based on the available information, MATCB is predicted to be persistent in water, soil and sediment, but is expected to have low bioaccumulation potential. The low importation quantity of this substance into Canada, along with information on physical and chemical properties and the use of the substance, indicate a low potential for releases into the Canadian environment. If released into the environment, it is expected that the substance will be mainly discharged to surface waters, where ultimately it will be transferred to sediment.

Based on data for similar azo dyes, MATCB is expected to have only a low to moderate potential for acute toxicity to aquatic organisms.

A predicted no-effect concentration (PNEC) was estimated based on the lowest nominal acute-effect concentration (EC_{50}) for Disperse Orange 30. The critical toxicity value was the 96-hr EC_{50} for *D. magna* of 5.8 mg/L (Table 9) based on nominal concentrations. A factor of 100 was then applied to account for extrapolating from acute to chronic (long-term) toxicity and from laboratory results for one species to other potentially sensitive species in the field. The resulting PNEC for MATCB is 0.058 mg/L.

For the principal exposure pathway resulting from down-the-drain releases through consumer uses, Mega Flush results estimate that PECs will not exceed the PNEC at any sites in Canada (i.e., the maximum risk quotients = 0.001) (Environment Canada 2008d). This indicates that down-the-drain consumer releases of MATCB are not expected to cause harm to aquatic organisms.

Therefore this substance is considered very unlikely to cause ecological harm in Canada.

Uncertainties in Evaluation of Ecological Risk

All modelling of a substance's physical and chemical properties and hazard characteristics is based on chemical structures. As MATCB is a UVCB, it cannot be represented by a single, discrete chemical structure. Therefore, for the purposes of modelling, a representative structure that would provide conservative estimates was identified.

Another area of uncertainty for MATCB is associated with the use of "read-across" physical and chemical properties, environmental fate data and toxicity data from analogues. This uncertainty is due to a lack of empirical data for monoazo dye analogues with similar functional groups. While the chemicals identified (Pigment Orange 61 and Disperse Orange 30) share some similarities with MATCB—being azo dyes with high molecular weights, similar cross-sectional diameters, having solid particulate structures that decompose at greater than 120°C (to 270°C), and being "dispersible" in water (i.e., not truly "soluble")—they do have differences in functional groups. These differences in chemical structure add uncertainty, because the properties, environmental fate and toxicity of MATCB may be somewhat different. However, it was reasoned that the similarities were sufficient (i.e., bioavailability potential) to include data from these analogues in the weight of evidence assessment of MATCB.

The persistence assessment is limited by the absence of biodegradation data, which necessitated the generation of model predictions. Although all model predictions have some degree of error, the biodegradation model outputs confirmed that MATCB is not likely to biodegrade quickly under oxic conditions and that MATCB meets the persistence criteria as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000). Nevertheless, it is clear that anaerobic degradation of the bioavailable portion of azo dyes in sediments to constitutive amines is much faster (half-lives in the order of days) than aerobic biodegradation. Although the amine degradation products are not expected to be biologically available because they form only in relatively deep anoxic sediment and can be irreversibly bound to sediment (Weber et al. 2001; Colon et al. 2002), this issue is a source of uncertainty in the toxicity assessment of MATCB.

The bioaccumulation assessment for MATCB was limited by the lack of empirical data and the inability of available models to reliably estimate bioaccumulation for azo dyes. Instead, the assessment relied on the use of bioaccumulation data for chemically similar azo substances.

Uncertainties are also present due to the lack of information on environmental concentrations in Canada for MATCB. The low quantity of MATCB available in Canada and the anticipated high removal rate in wastewater treatment plants suggest low releases of this substance into the Canadian environment.

The lack of experimental toxicity data for aquatic organisms is an additional source of uncertainty. However, based on the available data of similar azo substances and the

expected low water solubility of MATCB, this substance is not likely to be highly hazardous to aquatic organisms.

Also, regarding ecotoxicity, based on the predicted partitioning behaviour of this substance, the significance of soil and sediment as important media of exposure is not well addressed by the effects data available. Indeed, the only effects data identified apply primarily to pelagic aquatic exposures, although the water column may not be the medium of primary concern. Nevertheless, based on the relatively low aquatic toxicity of this substance, potential for harm to soil- and sediment-dwelling organisms is also expected to be low.

Potential to Cause Harm to Human Health

Exposure Assessment

No environmental measurements of MATCB were identified in the literature. Based on the release information, concentrations in environmental media are expected to be negligible.

Disperse dyes such as MATCB are used in the textile industry to colour synthetic fabrics such as polyesters and polyamides. Disperse dyes derive their name from the dyeing process employed (Danish EPA 1998). Because of their low water solubility, the dye compounds are typically milled to produce a fine powder and applied as a dispersion in water. The hydrophobic dye molecules adsorb to the hydrophobic textile, and heating induces uptake of the dye by the textile (Chudgar and Oakes 2003). MATCB does not form chemical bonds with the textile; therefore, migration is possible. MATCB may be used as a dyeing agent for synthetic fibres for personal apparel and domestic textile uses.

Upper-bounding exposure estimates were derived for two scenarios. The first scenario considered dermal exposure when an individual wears apparel made of a fabric dyed with MATCB, and the second considered exposure by the oral route for mouthing of the fabric by infants and young children. These are considered the most likely routes of exposure. The upper-bounding internal dose from dermal exposure to MATCB was estimated to range from 0.1 to 4 μg/kg-bw per day for all age groups wearing new, unwashed apparel possessing good to poor colourfastness properties (ETAD 2004). For infants and children, the estimated exposure via mouthing, was less than 0.1 μg/kg-bw per day. A recent study found that the amount of a disperse dye that migrated onto the skin of human volunteers was 300–600 times lower than that leached by sweat simulants (Meinke et al. 2009). This supports the conservative nature of the upper-bound exposure estimates. In addition, the dyes in textiles are expected to be leached out of fabric primarily by laundering, so any potential exposures would decline over time. Details of the assumptions used in these calculations are given in Appendix 3 and Appendix 4.

Health Effects Assessment

MATCB (CAS RN 106276-78-2) is a mixture of substances consisting of two substances, chemical 4 and chemical 5 as illustrated in Figure 1 as the major components. Also present in the mixture are Pigment Orange 61 (CAS RN 40716-47-0; chemical 6 in Figure 2) and the residual reactant CAS RN 43151-99-1 (Appendix 5). These substances may vary in proportion; therefore, all were considered in the search for available health effects information.

No empirical data were available with respect to the potential hazard of the four substances comprising MATCB. Therefore, the (Q)SAR models were used to predict the carcinogenicity and genotoxicity of these substances (CASETOX 2008; DEREK 2008; Leadscope 2009; and Toxtree 2009). A limited number of positive results for carcinogenicity were obtained, but the majority of the output was largely not in the domain of the models. Genotoxicity results were equivocal; for example, five positives and four negatives were returned for CAS RN 43151-99-1 (Appendix 6). As only limited (Q)SAR data were available with respect to the potential toxicity of MATCB, relevant information on analogues of MATCB and potential azo cleavage products was also considered.

One structural analogue of MATCB for the purpose of read-across for human health information was identified. 4,4′-Diaminoazobenzene (DAAB or 4,4′-azoaniline) (CAS RN 538-41-0) was identified as an analogue of the MATCB residual reactant CAS RN 43151-99-1. DAAB was mutagenic with S9 activation in *Salmonella typhimurium* strains TA98 and TA1538, negative with S9 activation in strains TA100, TA1535 and TA1537, and negative without S9 activation in strains TA98, TA100, TA1535, TA1537 and TA1538 (Shahin 1989). Additionally, carcinogenicity was not observed in male and female BALB/c mice consuming DAAB daily at up to 600 mg/kg diet for 60 weeks (mice were followed to 140 weeks) (Della Porta and Dragani 1981).

Since MATCB is a member of the family of azo substances, relevant health effects information on its potential azo cleavage products was also considered. It has been demonstrated that certain azo substances can undergo metabolism by reductive cleavage mediated by azoreductase enzymes found in mammalian tissues as well as bacteria of the intestine and skin (Platzek et al. 1999; Golka et al. 2004; Chen 2006; Xu et al. 2007; Stingley et al. 2010). While it is recognized that the degree of azo reduction is likely influenced by various factors (e.g., solubility of the parent substance, presence and position of molecular substituents), in the absence of chemical-specific metabolism data, it is assumed that exposure to an azo substances may also lead to exposure to its corresponding azo cleavage products, typically aromatic amines.

Therefore, the potential azo cleavage products of the four substances comprising MATCB; namely, toluene-2,5-diamine (CAS RN 95-70-5), *p*-phenylenediamine (PPD) (CAS RN 106-50-3), CAS RN 5590-19-2 and a fourth metabolite (CAS RN not assigned), here referred to as “M4”, are also considered in this screening assessment (Appendix 5).

Since empirical toxicological data were not available for two of the potential azo cleavage products (CAS RN 5590-19-2 and 4-M), (Q)SAR modelling predictions were therefore used to identify possible hazards associated with these substances. For genotoxicity, three positives, two negatives and four "not in the domain" were obtained for CAS RN 5590-19-2, and four positives and five "not in the domain" were obtained for M4 (Appendix 6).

The empirical health effects data identified for the other potential azo cleavage products of MATCB, PPD and toluene-2,5-diamine, are summarized below.

The European Commission's Scientific Committee on Consumer Products (SCCP) recently conducted risk assessments on PPD (SCCP 2006) and toluene-2,5-diamine (SCCP 2007). Regarding PPD toxicity, the SCCP reported the opinion of the Scientific Committee on Cosmetology (SCC) that was generated in 1991. The SCC considered PPD as having moderate acute oral toxicity and low dermal toxicity and identified PPD as a skin sensitizer (SCCP 2006). PPD is currently listed on Health Canada's Cosmetic Ingredient Hotlist, not permitting in products intended for use on the skin (Health Canada 2009). *In vitro* genotoxicity data for PPD are mixed, with both negative and positive results in the Ames assay, the mouse lymphoma assay and the micronucleus test assessing mammalian cell clastogenicity. Positive results were also seen for chromosomal aberration in CHO-K1 cells. However, PPD was negative *in vivo* for bone marrow clastogenicity and for hepatocyte unscheduled deoxyribonucleic acid (DNA) synthesis (UDS); it did not damage DNA in the comet assay, and it did not bind to liver DNA. Subchronic feeding studies in mice and rats following Test Guideline 408 of the Organisation for Economic Co-operation and Development (OECD) established a no-observed-adverse-effect level (NOAEL) of 4 mg/kg-bw per day based on increased liver and kidney weights. A draft report of another study reported NOAELs of 5 and 10 mg/kg-bw per day for maternal and developmental toxicity in Sprague-Dawley rats, respectively. Multiple carcinogenicity studies were identified, including a chronic feeding study in B6C3F1 mice and F344 rats conducted by the US National Toxicology Program (NTP) in 1979. This study provided evidence that PPD is not carcinogenic (SCCP 2006).

The SCCP assessment of toluene-2,5-diamine reported positive *in vitro* genotoxicity results (Ames assay with S9 activation in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537; mammalian cell clastogenicity and hepatocyte UDS), negative *in vitro* results (Ames assay without S9 activation; mammalian cell gene mutation [*tk* locus]) and negative *in vivo* results (mouse bone marrow micronucleus test; hepatocyte UDS; dominant lethal assay). Subchronic feeding studies in mice and rats following OECD Test Guideline 408 established a NOAEL of 1.4 mg/kg-bw per day based on increased serum aspartate transaminase and increased liver and kidney weights, respectively. NOAELs of 45 mg/kg-bw per day for reproductive toxicity in Sprague-Dawley rats and 50 mg/kg-bw per day for embryotoxicity and teratogenicity in New Zealand White rabbits were established in separate studies. An oral carcinogenicity study conducted by the US National Institute of Health in 1978 did not show an unequivocal carcinogenic effect of the substance in B6C3F1 mice or F344 rats (SCCP 2007).

Characterization of Risk to Human Health

No empirical data were identified for the substances comprising MATCB. Results from (Q)SAR predictions for genotoxicity were equivocal, and the limited results obtained for carcinogenicity were mixed. Information from one parental substance analogue and two potential azo cleavage products (PPD and toluene-2,5-diamine) was equivocal for genotoxicity and essentially negative for carcinogenicity. There are therefore limited data suggestive of a potential hazard for MATCB. However, the limited health effects information available precludes selection of a critical effect level for use in risk characterization of this substance. .

The potential for exposure of the general population to MATCB from environmental media is expected to be negligible. Exposure to MATCB from the wearing of dyed personal apparel by all age groups and from incidental mouthing of fabrics by children has been quantified and is low.

As exposure of the general population in Canada based on the use of the substance as a synthetic textile dye is expected to be low to negligible, the risk to human health is considered to be low.

Uncertainties in Evaluation of Risk to Human Health

There are uncertainties associated with the exposure assessment. Substance-specific information such as migration factors and solubility was not available. The sources of exposure have been broadly characterized as synthetic fabrics because no specific consumer products were identified. However, confidence is high in the conservative nature of the exposure estimates, because the migration factor used in the assessment corresponds to daily exposure to new, unwashed fabrics with poor colourfastness, while leaching is expected to occur primarily during laundering. Additionally, there are empirical data demonstrating that the amount of a disperse dye that migrates onto human volunteers is substantially lower than that leaching into solution.

Confidence in the toxicity database is considered very low, as there were no empirical data available for MATCB, as well as a lack of information on the potential for this substance to undergo azo cleavage, a primary consideration when evaluating the toxicity of azo compounds. There is additional uncertainty given that MATCB is a mixture in which the proportion of each parental substance may vary.

Conclusion

Based on the information presented in this screening assessment, it is concluded that MATCB 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.

While empirical toxicological data on one analogue and two potential azo cleavage products provide an indication of a potential hazard for MATCB, based upon consideration of the limited health effects information and low to negligible exposure of the general population to MATCB, it is concluded that MATCB is not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

It is therefore concluded that MATCB does not meet any of the criteria in section 64 of CEPA 1999. Additionally, MATCB meets the criteria for persistence but not the criteria for bioaccumulation as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Because this substance is listed on the Domestic Substances List, its import and manufacture in Canada are not subject to notification under subsection 81(1) of CEPA 1999. Given the potential hazardous properties of this substance, there is concern that new activities that have not been identified or assessed could lead to this substance meeting the criteria set out in section 64 of the Act. Therefore, it is recommended that the Domestic Substances List be amended, under subsection 87(3) of the Act, to indicate that subsection 81(3) of the Act applies with respect to the substance so that new manufacture, import or use of this substance is notified and undergoes ecological and human health risk assessments.

In addition and where relevant, research and monitoring will support verification of assumptions used during the screening assessment.

Considerations for Follow-up

MATCB belongs to a group of azo substances that may metabolize to aromatic amines, which as a chemical class are known to exhibit hazardous properties, including carcinogenicity. Therefore, additional activity (e.g., research, monitoring and surveillance, assessment) to characterize the risk to human health in Canada of this broader group of azo substances may be undertaken.

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Appendix 1 - Robust Study Summary

Robust Study Summaries Form and Instructions: Aquatic B				
No	**Item**	**Weight**	**Yes/No**	**Specify**
1	**Reference:** Shen, Genxiang and Hu, Shuangqing. 2008. Bioconcentration Test of C.I. Disperse Orange 30 in Fish. Prepared by Environmental Testing Laboratory, Shanghai Academy of Environmental Sciences, Shanghai, China for Dystar in the name of Ecological and Toxicological Association of the Dyes and Organic Pigments Manufacturers (ETAD) Basel, Switzerland. Report No. S-070-2007. Submitted to Environment Canada in April 2008. Challenge Submission ID#8351.			
2	Substance identity: CAS RN	n/a	Y	5261-31-4
3	Substance identity: chemical name(s)	n/a	Y	Propanenitrile, 3-[[2-(acetyloxy)ethyl][4-[(2,6-dichloro-4-nitrophenyl)azo]phenyl]amino]-
4	Chemical composition of the substance	2	N	
5	Chemical purity	1	N	
6	Persistence/stability of test substance in aquatic solution reported?	1	N	
7	If test material is radiolabelled, were precise position(s) of the labelled atom(s) and the percentage of radioactivity associated with impurities reported?	2	n/a	
Method				
8	Reference	1	Y	OECD guidelines for the testing of chemicals No. 305B-1996
9	OECD, EU, national, or other standard method?	3	Y	OECD
10	Justification of the method/protocol if a non-standard method was used	2		
11	GLP (good laboratory practice)	3	N	
Test organism				
12	Organism identity: name	n/a	Y	Zebra fish (*Brachydanio rerio*)
13	Latin or both Latin and common names reported?	1	Y	Both
14	Life cycle age/stage of test organism	1	N	
15	Length and/or weight	1	Y	Mean body length 3.91 ± 0.18 cm and mean body weight 0.32 ± 0.06 g
16	Sex	1	N	
17	Number of organisms per replicate	1	Y	7
18	Organism loading rate	1	Y	20 mg/L
19	Food type and feeding periods during the acclimation period	1	Y	Fed a commercial fish diet until one day before start of test
Test design/conditions				
20	Experiment type (laboratory or field)	n/a	Y	Laboratory
21	Exposure pathways (food, water, both)	n/a	Y	Water
22	Exposure duration	n/a	Y	28 days
23	Number of replicates (including controls)	1	Y	
24	Concentrations	1	Y	20 mg/L
25	Food type/composition and feeding periods during the test	1	Y	Fish were fed two hours before water renewal

26	If BCF/BAF derived as a ratio of chemical concentration in the organism and in water, was experiment duration equal to or longer than the time required for the chemical concentrations to reach steady state?	3	Y	28 days
27	If BCF/BAF derived as a ratio of chemical concentration in the organism and in water, were measured concentrations in both water and organism reported?	3	Y	
28	Were concentrations in the test water measured periodically?	1	Y	On three separate days
29	Were the exposure media conditions relevant to the particular chemical reported? (e.g., for metal toxicity - pH, DOC/TOC, water hardness, temperature)	3	Y	Yes, every second day
30	Photoperiod and light intensity	1	Y	12:12
31	Stock and test solution preparation	1	Y	
32	Analytical monitoring intervals	1	Y	Every 2nd day for dissolved oxygen, pH and temperature
33	Statistical methods used	1	Y	
34	Was solubilizer/emulsifier used if the chemical was unstable or poorly soluble?	n/a	N	
Information relevant to the data quality				
35	Was the test organism relevant to the Canadian environment?	3	Y	
36	Were the test conditions (pH, temperature, DO, etc.) typical for the test organism?	1	Y	
37	Do system type and design (static, semi-static, flow-through; sealed or open; etc.) correspond to the substance's properties and the organism's nature/habits?	2	Y	Semi-static
38	Was pH of the test water within the range typical for the Canadian environment (6 to 9)?	1	Y	7.22–7.84
39	Was temperature of the test water within the range typical for the Canadian environment (5 to 27°C)?	1	Y	22–23
40	Was lipid content (or lipid-normalized BAF/BCF) reported?	2	Y	
41	Were measured concentrations of a chemical in the test water below the chemical's water solubility?	3	N	
42	If radiolabelled test substance was used, was BCF determination based on the parent compound (i.e., not on total radiolabelled residues)?	3	n/a	
Results				
43	Endpoints (BAF, BCF) and values	n/a	n/a	BCF
44	Was BAF or BCF determined as: 1) the ratio of chemical concentration in the organism and in water, or 2) the ratio of the chemical uptake and elimination rate constants?	n/a	n/a	1
45	Was BAF/BCF derived from a 1) tissue sample or 2) whole organism?	n/a	n/a	2
46	Was 1) average or 2) maximum BAF/BCF used?	n/a	n/a	1
47	***Score*: ... %**	***75.0***		
48	***Environment Canada reliability code*:**	***2***		
49	***Reliability category (high, satisfactory, low)*:**	***Satisfactory Confidence***		

50	***Comments***	***The present procedure is based on semi-static conditions (renewal of test solutions every 2 days). Therefore, test chemicals with very low water solubility, e.g., Disperse Orange 30, can also be characterized as to their bioconcentration potential without adding solvents or other auxiliary substances that may affect the results.***

Robust Study Summary Form: Aquatic iT				
No	**Item**	**Weight**	**Yes/No**	**Specify**
1	Reference: Sandoz 1975. Acute fish toxicity (Rainbow trout) 48 hr			
2	Substance identity: CAS RN	n/a	Y	5261-31-4
3	Substance identity: chemical name(s)	n/a	Y	
4	Chemical composition of the substance	2	N	
5	Chemical purity	1	N	
6	Persistence/stability of test substance in aquatic solution reported?	1	N	
Method				
7	Reference	1	Y	
8	OECD, EU, national or other standard method?	3	Y	
9	Justification of the method/protocol if a non-standard method was used	2		
10	GLP (good laboratory practice)	3	Y	
Test organism				
11	Organism identity: name	n/a	Y	Rainbow trout
12	Latin or both Latin and common names reported?	1	Y	
13	Life cycle age/stage of test organism	1	N	
14	Length and/or weight	1	Y	
15	Sex	1	N	
16	Number of organisms per replicate	1	N	
17	Organism loading rate	1	N	
18	Food type and feeding periods during the acclimation period	1	N	
Test design/conditions				
19	Test type (acute or chronic)	n/a	Y	Acute
20	Experiment type (laboratory or field)	n/a	Y	Laboratory
21	Exposure pathways (food, water, both)	n/a		
22	Exposure duration	n/a	Y	48 hr
23	Negative or positive controls (specify)	1	N	
24	Number of replicates (including controls)	1	N	
25	Nominal concentrations reported?	1	N	

26	Measured concentrations reported?	3	N	
27	Food type and feeding periods during the long-term tests	1	N	
28	Were concentrations measured periodically (especially in the chronic test)?	1	N	
29	Were the exposure media conditions relevant to the particular chemical reported? (e.g., for metal toxicity – pH, DOC/TOC, water hardness, temperature)	3	N	
30	Photoperiod and light intensity	1	N	
31	Stock and test solution preparation	1	N	
32	Was solubilizer/emulsifier used if the chemical was poorly soluble or unstable?	1	N	
33	If solubilizer/emulsifier was used, was its concentration reported?	1		
34	If solubilizer/emulsifier was used, was its ecotoxicity reported?	1		
35	Analytical monitoring intervals	1	N	
36	Statistical methods used	1	N	
Information relevant to the data quality				
37	Was the endpoint directly caused by the chemical's toxicity, not by the organism's health (e.g., when mortality in the control > 10%) or physical effects (e.g., "shading effect")?	n/a		
38	Was the test organism relevant to the Canadian environment?	3	Y	
39	Were the test conditions (pH, temperature, DO, etc.) typical for the test organism?	1	N	
40	Do system type and design (static, semi-static, flow-through; sealed or open; etc.) correspond to the substance's properties and the organism's nature/habits?	2	N	
41	Was pH of the test water within the range typical for the Canadian environment (6 to 9)?	1	N	
42	Was temperature of the test water within the range typical for the Canadian environment (5 to 27°C)?	1	Y	
43	Was toxicity value below the chemical's water solubility?	3	N	
Results				
44	Toxicity values (specify endpoint and value)	n/a	n/a	48 hr LC_{50} > 700 mg/L

45	Other endpoints reported - e.g., BCF/BAF, LOEC/NOEC (specify)?	n/a		
46	Other adverse effects (e.g., carcinogenicity, mutagenicity) reported?	n/a		
47	***Score*: ... %**	***28.9***		
48	***Environment Canada reliability code*:**	***4***		
49	***Reliability category (high, satisfactory, low)*:**	***Not Satisfactory***		
50	***Comments***			

Robust Study Summary Form: Aquatic iT				
No	**Item**	**Weight**	**Yes/No**	**Specify**
1	Reference: Environment Canada. 1995. NSN submission.			
2	Substance identity: CAS RN	n/a	N	
3	Substance identity: chemical name(s)	n/a	Y	
4	Chemical composition of the substance	2	N	
5	Chemical purity	1	N	
6	Persistence/stability of test substance in aquatic solution reported?	1	N	
Method				
7	Reference	1	Y	OECD 203
8	OECD, EU, national, or other standard method?	3	Y	
9	Justification of the method/protocol if a non-standard method was used	2		not applicable
10	GLP (good laboratory practice)	3	Y	
Test organism				
11	Organism identity: name	n/a	Y	Rainbow trout
12	Latin or both Latin and common names reported?	1	Y	
13	Life cycle age/stage of test organism	1	Y	Mean length 51 mm and mean weight 1.54 g
14	Length and/or weight	1	Y	See above
15	Sex	1		Not applicable
16	Number of organisms per replicate	1	Y	10
17	Organism loading rate	1	Y	
18	Food type and feeding periods during the acclimation period	1	Y	
Test design/conditions				
19	Test type (acute or chronic)	n/a	Y	Acute
20	Experiment type (laboratory or field)	n/a	y	Lab
21	Exposure pathways (food, water, both)	n/a	y	Water
22	Exposure duration	n/a	y	96 hr
23	Negative or positive controls (specify)	1	Y	3
24	Number of replicates (including controls)	1	Y	2
25	Nominal concentrations reported?	1	Y	320 to 3200 mg/L
26	Measured concentrations reported?	3	N	
27	Food type and feeding periods during the long-term tests	1		Not applicable

28	Were concentrations measured periodically (especially in the chronic test)?	1	N	
29	Were the exposure media conditions relevant to the particular chemical reported? (e.g., for metal toxicity - pH, DOC/TOC, water hardness, temperature)	3	Y	
30	Photoperiod and light intensity	1	Y	
31	Stock and test solution preparation	1	Y	
32	Was solubilizer/emulsifier used if the chemical was poorly soluble or unstable?	1	N	
33	If solubilizer/emulsifier was used, was its concentration reported?	1		
34	If solubilizer/emulsifier was used, was its ecotoxicity reported?	1		
35	Analytical monitoring intervals	1	Y	
36	Statistical methods used	1	Y	
Information relevant to the data quality				
37	Was the endpoint directly caused by the chemical's toxicity, not by the organism's health (e.g., when mortality in the control >10%) or physical effects (e.g., "shading effect")?	n/a	Y	
38	Was the test organism relevant to the Canadian environment?	3	Y	
39	Were the test conditions (pH, temperature, DO, etc.) typical for the test organism?	1	Y	
40	Do system type and design (static, semi-static, flow-through; sealed or open; etc.) correspond to the substance's properties and the organism's nature/habits?	2	Y	
41	Was pH of the test water within the range typical for the Canadian environment (6 to 9)?	1	Y	
42	Was temperature of the test water within the range typical for the Canadian environment (5 to 27°C)?	1	Y	
43	Was toxicity value below the chemical's water solubility?	3		Unknown water solubility
Results				
44	Toxicity values (specify endpoint and value)	n/a	n/a	96-hr LC_{50}
45	Other endpoints reported - e.g., BCF/BAF, LOEC/NOEC (specify)?	n/a	N	
46	Other adverse effects (e.g., carcinogenicity, mutagenicity) reported?	n/a	N	
47	***Score*: ... %**	***77.5***		
48	***Environment Canada reliability code*:**	***2***		
49	***Reliability category (high, satisfactory, low)*:**	***Satisfactory Confidence***		
50	***Comments***			

Robust Study Summary Form: Aquatic iT				
No	**Item**	**Weight**	**Yes/No**	**Specify**
1	Reference: Safepharm Laboratories Ltd. 1990. Acute toxicity to rainbow trout. Project number 47/781.			
2	Substance identity: CAS RN	n/a	Y	5261-31-4

3	Substance identity: chemical name(s)	n/a	Y	
4	Chemical composition of the substance	2	N	
5	Chemical purity	1	N	
6	Persistence/stability of test substance in aquatic solution reported?	1	N	
Method				
7	Reference	1	N	
8	OECD, EU, national or other standard method?	3	N	
9	Justification of the method/protocol if a non-standard method was used	2	N	
10	GLP (good laboratory practice)	3		n/a
Test organism				
11	Organism identity: name	n/a		Rainbow trout
12	Latin or both Latin and common names reported?	1	Y	
13	Life cycle age/stage of test organism	1	Y	
14	Length and/or weight	1	Y	
15	Sex	1		n/a
16	Number of organisms per replicate	1	Y	3 to 10
17	Organism loading rate	1	Y	0.70 g body weight/L
18	Food type and feeding periods during the acclimation period	1		n/a since acute test
Test design/conditions				
19	Test type (acute or chronic)	n/a		Acute
20	Experiment type (laboratory or field)	n/a		Lab
21	Exposure pathways (food, water, both)	n/a		Water
22	Exposure duration	n/a		96 hr
23	Negative or positive controls (specify)	1	Y	Positive
24	Number of replicates (including controls)	1	Y	2 at definitive study
25	Nominal concentrations reported?	1	Y	3
26	Measured concentrations reported?	3	N	
27	Food type and feeding periods during the long-term tests	1		n/a
28	Were concentrations measured periodically (especially in the chronic test)?	1	N	
29	Were the exposure media conditions relevant to the particular chemical reported? (e.g., for metal toxicity - pH, DOC/TOC, water hardness, temperature)	3	Y	
30	Photoperiod and light intensity	1	N	
31	Stock and test solution preparation	1	N	
32	Was solubilizer/emulsifier used if the chemical was poorly soluble or unstable?	1	N	
33	If solubilizer/emulsifier was used, was its concentration reported?	1		n/a
34	If solubilizer/emulsifier was used, was its ecotoxicity reported?	1		n/a
35	Analytical monitoring intervals	1	Y	
36	Statistical methods used	1	N	
Information relevant to the data quality				
37	Was the endpoint directly caused by the chemical's toxicity, not by the organism's health (e.g., when mortality in the control > 10%) or physical effects (e.g., "shading effect")?	n/a	Y	

38	Was the test organism relevant to the Canadian environment?	3	Y	
39	Were the test conditions (pH, temperature, DO, etc.) typical for the test organism?	1	Y	
40	Do system type and design (static, semi-static, flow-through; sealed or open; etc.) correspond to the substance's properties and the organism's nature/habits?	2		n/a
41	Was pH of the test water within the range typical for the Canadian environment (6 to 9)?	1	N	No pH given
42	Was temperature of the test water within the range typical for the Canadian environment (5 to 27°C)?	1	Y	
43	Was toxicity value below the chemical's water solubility?	3	N	Water solubility for this substance was 0.07
Results				
44	Toxicity values (specify endpoint and value)	n/a		96 hr LC_{50} > 100 mg/L
45	Other endpoints reported - e.g., BCF/BAF, LOEC/NOEC (specify)?	n/a	N	
46	Other adverse effects (e.g., carcinogenicity, mutagenicity) reported?	n/a	N	
47	***Score*: ... %**	***43.6***		
48	***Environment Canada reliability code*:**	***3***		
49	***Reliability category (high, satisfactory, low)*:**	***Low Confidence***		
50	***Comments***			

Appendix 2 – PBT Model Inputs Summary Table

Most models are not suitable for MATCB, as it is an azo dye. Only the EPI SUITE (BIOWIN) and CPOPs (CATABOL) models have been applied, using SMILES input for the subject chemical to predict bioaccumulation.

Representative SMILES for MATCB: c1cc(N)ccc1N=Nc2c(C)cc(cc2)NC3=NC(=O)c4c(Cl)c(Cl)c(Cl)c(Cl)c34

	Physical and Chemical Fate	**Fate**	**Fate**	**Fate**	**Fate**	**Fate**	**Fate**	**PBT Profiling**	**Ecotoxicity**
Model input parameters	EPI Suite (all models, including AOPWIN, KOCWIN, BCFWIN BIOWIN and ECOSAR)	STP (1) ASTreat (2) SimpleTreat (3) (required inputs differ depending on model)	EQC (required inputs differ depending on chemical - Type I vs. Type II chemical)	TaPL3 (required inputs depending on chemical - Type 1 vs. Type 2 chemical)	OECD POPs Tool	Arnot-Gobas BCF/BAF model	Gobas Wolf BMF model	Canadian POPs (including: Catabol, BCF Mitigating Factors Model, OASIS Toxicity Model)	Artificial Intelligence Expert System (AIEPS) / TOPKAT/ ASTER
SMILES code	x (BIOWIN only)	n/a	n/a	n/a	n/a	n/a	n/a	x (CATABOL only)	n/a

Appendix 3: Upper-bounding estimates of exposure to MATCB from textiles

Consumer product scenario	Upper-bounding estimates of exposure (µg/kg-bw per day) to MATCB for various age groups[1]				
	0–6 months[2]	0.5–4 years[3]	5–11 years[4]	12–19 years[5]	20+ years[6]
Dermal: wearing of personal apparel	0.2–4	0.2–3	0.2–3	0.1–2	0.1–2
Oral: mouthing of personal apparel	0.1	0.06	NA	NA	NA

NA, not applicable

[1] Upper-bounding leachable fraction was estimated to range from 0.03% for colourfast textiles (ETAD 2004) to 0.5% for textiles with poor colourfastness (Kraetke and Platzek 2005).

[2] Assumed to weigh 7.5 kg, have a body surface area (excluding head and hands) of 0.28 m^2 (Health Canada 1998) and spend 23 min/day mouthing (Norris and Smith 2002).

[3] Assumed to weigh 15.5 kg, have a body surface area (excluding head and hands) of 0.46 m^2 (Health Canada 1998) and spend 29 min/day mouthing (Norris and Smith 2002).

[4] Assumed to weigh 31.0 kg and have a body surface area (excluding head and hands) of 0.80 m^2 (Health Canada 1998).

[5] Assumed to weigh 59.4 kg and have a body surface area (excluding head and hands) of 1.4 m^2 (Health Canada 1998).

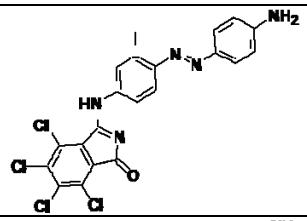
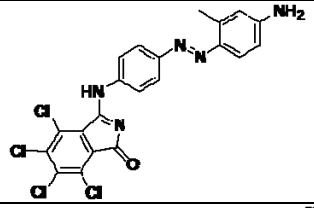
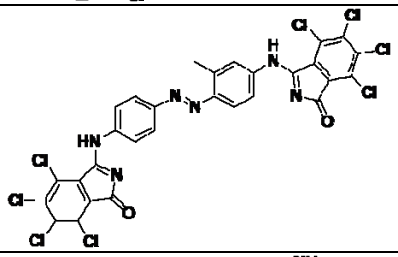
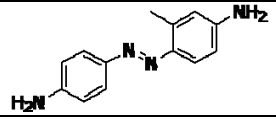
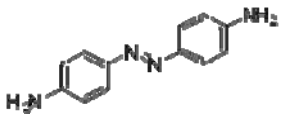
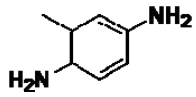
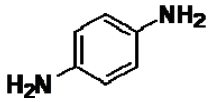
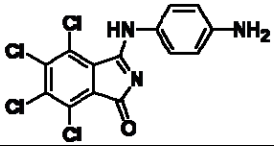
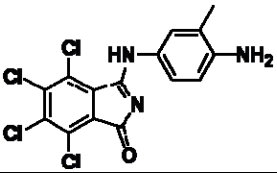
[6] Assumed to weigh 70.9 kg and have a body surface area (excluding head and hands) of 1.6 m^2 (Health Canada 1998).

Appendix 4: Upper-bounding estimated exposure calculations for infants aged 0–6 months

Consumer product scenario	Assumptions	Upper-bounding estimated exposure
Wearing of dyed personal apparel made from synthetic fabrics	Exposure scenario: ConsExpo 4.0, direct dermal contact with product: migration (RIVM 2005). Example for infants aged 0–6 months. Concentration: 1% by weight (Kraetke and Platzek 2005) Fabric density: 100 g/m^2 (Kraetke and Platzek 2005) **General assumptions** - Exposure frequency: 365 times/year - Body weight: 7.5 kg (Health Canada 1998) - Body surface area, excluding head and hands[1]: 0.28 m^2 (Health Canada 1998) **Dermal route** - Exposed area[1]: 0.28 m^2 (Health Canada 1998) - Leachable fraction: 0.5% (Kraetke and Platzek 2005) - Product amount[2]: 0.28 g - Skin contact factor: 1 (fraction) - Uptake fraction: 2% (Kraetke and Platzek 2005)	**Dermal chronic** Internal dose = 0.004 mg/kg-bw per day
Mouthing of dyed fabrics	Exposure is estimated below for infants aged 0–6 months. The estimated daily intake for ingestion from mouthing: $= \frac{WS \times V_s \times CF \times FR \times AF_o \times EF}{BW}$ where: *WS* = water solubility of MATCB (read-across for azo dyes) = 35.5 mg/L (Baughman and Perenich 1988) V_s = salivary flow rate = 0.22 mL/min (Environ 2003a, b) *CF* = conversion factor to convert L to mL = 0.001 L/mL *FR* = fractional extraction by saliva = 0.5% (ETAD 1983)[3] AF_o = absorption factor by oral route = 1 *EF* = exposure frequency of mouthing behaviour = 23 min/day (Norris and Smith 2002) *BW* = body weight = 7.5 kg (infants aged 0–6 months) (Health Canada 1998) = (35.5 mg/L × 0.22 mL/min × 0.001 L/mL × 0.005 × 1 × 23 min/day) / 7.5 kg = 0.0001 mg/kg-bw per day	**Oral chronic** Internal dose = 0.0001 mg/kg-bw per day

[1] This is assumed to equal the amount of fabric in contact with the skin.
[2] Product amount = fabric density × amount of fabric × concentration = (100 g/m^2) × (0.28 m^2) × (0.01) = 0.28 g.
[3] Maximum amount of dye extracted by simulated saliva from child-oriented synthetic textiles after 4 h was 0.13%; 0.5% is used to represent an upper bound.

Appendix 5: Structures and data considered in characterization of human health effects

Substance Identification	Structure	Data considered/available
Chemical 4 (Figure 1) CAS RN 106276-78-2 (parent)		(Q)SAR
Chemical 5 (Figure 1) CAS RN 106276-78-2 (parent)		(Q)SAR
Chemical 6 (Figure 2) CAS RN 40716-47-0 (parent)		(Q)SAR
Chemical 3 (Figure 1) CAS RN 43151-99-1 (residual reactant)		(Q)SAR Analogue read-across
DAAB CAS RN 538-41-0 (analogue of CAS RN 43151-99-1)		Ames assay Chronic feeding study in mice
Toluene-2,5-diamine CAS RN 95-70-5 (possible metabolite)		International risk assessment (SCCP 2007)
PPD CAS RN 106-50-3 (possible metabolite)		International risk assessment (SCCP 2006)
CAS RN 5590-19-2 (possible metabolite)		(Q)SAR
M4 (possible metabolite)		(Q)SAR

Appendix 6. Summary of (Q)SAR results for MATCB and potential MATCB metabolites

Carcinogenicity

CAS RN (structures corresponding to Figures 1 and 2)	Derek[1]	Toxtree[2]		Model Applier[3]				Casetox[4]			
	Cancer	SA gtx	Cancer QSAR	m-rat	f-rat	m-mice	f-mice	m-rat	f-rat	m-mice	f-mice
43151-99-1 (chemical 3)	P	P	N	P[5]	ND	ND	N	N	N	N	N
40716-47-0 (chemical 6)	P	P	N	ND	ND	ND	ND	ND	ND	ND	ND
106276-78-2(chemical 5)	P	P	N	ND	ND	ND	ND	ND	ND	ND	ND
5590-19-2	P	P	N	ND	ND	ND	ND	ND	ND	ND	ND
106276-78-2(chemical 4)	P	P	N	ND	ND	ND	ND	ND	ND	ND	ND
M4 (no CAS RN)	P	P	N	ND	ND	ND	ND	ND	ND	ND	ND

Genotoxicity

CAS RN (structures corresponding to Figures 1 and 2)	Ames				ChrAb		Micronuclei induction		
	Derek	TT[6]	MA	CT	MA	CT[7]	TT	MA	CT
43151-99-1 (chemical 3)	P	P	P	N	N	P	P	N	N
40716-47-0 (chemical 6)	P	P	N	N	N	ND	P	ND	N
106276-78-2(chemical 5)	P	P	IC	N	ND	N	P	ND	ND
5590-19-2	P	P	N	N	ND	ND	P	ND	ND
106276-78-2(chemical 4)	P	P	IC	N	N	ND	P	ND	ND
4-M (no CAS RN)	P	P	ND	P	ND	ND	P	ND	ND

CAS RN, Chemical Abstracts Service Registry Number; ChrAb, chromosomal aberration; CT, Casetox; f, female; IC, inconclusive; m, male; MA, Model Applier; N, negative; ND, not in domain of model; SA gtx, structural alert for genotoxic carcinogen; P, positive; TT, Toxtree

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