Screening Assessment for the Challenge

2-Naphthalenesulfonic acid, 7-[[4,6-bis[[3-(diethylamino)propyl]amino]-1,3,5-triazin-2-yl]amino]-4-hydroxy-3-[[4-(phenylazo)phenyl]azo]-, monoacetate (salt)

Chemical Abstracts Service Registry Number
71032-95-6

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 2-naphthalenesulfonic acid, 7-[[4,6-bis[[3-(diethylamino)propyl]amino]-1,3,5-triazin-2-yl]amino]-4-hydroxy-3-[[4-(phenylazo)phenyl]azo]-, monoacetate (salt) (NDTHPM), Chemical Abstracts Service Registry Number 71032-95-6. This substance was identified as a high priority for screening assessment and included in the Challenge because it had been found to meet the ecological categorization criteria for persistence, bioaccumulation potential and inherent toxicity to non-human organisms and is believed to be in commerce in Canada. NDTHPM 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.

NDTHPM is a synthetic cationic direct (azo) dye that is used as a colorant primarily for paper. The substance does not naturally occur in the environment. As a result of an industry survey conducted pursuant to section 71 of CEPA 1999, no companies reported manufacturing or using NDTHPM in Canada above reporting thresholds in 2006. However, data that were submitted confirmed that this substance was imported into Canada in a total quantity between 100 and 1000 kg in both 2005 and 2006. The quantity of NDTHPM imported into Canada, along with its intended use, indicate that it could be released into the Canadian environment.

Based on reported use patterns and certain assumptions related to dyes in general, a large proportion of the substance is predicted to end up in landfills or be incinerated (~37%). Approximately 11% is estimated to be released to wastewater, while the remaining mass (~52%) is estimated to be transferred to recycling activities. NDTHPM is anticipated to be highly soluble in water, based on data for a structural analogue. However, given the positive and negative charges that exist on the molecule in solution (pH 6–9), like other ionic dyes, it will have an affinity for solid particles due to electrostatic interactions. Thus, it may settle in bed sediments to some degree and if present in soil, will tend to remain there.

Predicted data suggest that NDTHPM will persist in aerobic environments (water, soil and sediment). Degradation of azo dyes under anaerobic or reducing conditions may occur relatively rapidly, but would be limited to specific environments (e.g., deep layers of sediments), with potentially harmful metabolites being formed as a result of cleavage of its azo bonds. However, in these situations, exposure to aquatic organisms would be limited. The high water solubility as well as other physical and chemical properties (e.g., low experimental log K_{ow}) of its structural analogue, and the charged nature and large molecular size of the substance itself, suggest that NDTHPM has a low potential to accumulate in the lipid tissues of organisms. Therefore, this substance meets the persistence criteria but does not meet the bioaccumulation criteria as set out in the *Persistence and Bioaccumulation Regulations*. In addition, experimental acute and

chronic aquatic toxicity values for NDTHPM and a structural analogue indicate that this substance is not expected to cause acute harm to aquatic organisms at low concentrations.

For this screening assessment, a conservative exposure scenario was selected in which an industrial operation discharges NDTHPM into the aquatic environment through a single wastewater treatment plant. The upper end of the reporting range of 1000 kg was used to conservatively estimate release and exposure levels. The predicted environmental concentration in water of this substance was below the predicted no-effect concentration for sensitive aquatic organisms, resulting in a conservative risk quotient much lower than 1.

No empirical health effects information was identified for NDTHPM. The outputs of quantitative structure-activity relationship (QSAR) predictions for genotoxicity and carcinogenicity were mixed. Information from aromatic amines potentially generated from cleavage of the azo bonds in NDTHPM suggests a potential concern for genotoxicity and carcinogenicity. The potential for exposure of the general population to NDTHPM from environmental media is expected to be negligible. There is no expected exposure to NDTHPM from food. Exposure to NDTHPM from consumer products (e.g., non-food paper products containing NDTHPM dye) is expected to be negligible by dermal route of exposure and low for incidental events such as mouthing by toddlers. Although the potential hazard of NDTHPM is recognized, as exposure of the general population in Canada based on the use of the substance as a paper dye is expected to be low to negligible, the risk to human health is considered to be low.

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

Based on the information available, it is concluded that NDTHPM is not a substance entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

Based on the information available, it is concluded that NDTHPM 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 hazardous properties of this substance, there is concern that new activities that have not been identified or assessed could lead to the 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 this substance so that new manufacture, import or use of the substance is notified and undergoes ecological and human health risk assessments.

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

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, 2-naphthalenesulfonic acid, 7-[[4,6-bis[[3-(diethylamino)propyl]amino]-1,3,5-triazin-2-yl]amino]-4-hydroxy-3-[[4-(phenylazo)phenyl]azo]-, monoacetate (salt), 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 March 14, 2009 (Canada 2009a, 2009b). A substance profile was released at the same time. The substance profile presented the technical information available prior to December 2005 that formed the basis for categorization of this substance. As a result of the Challenge, submissions of information pertaining to the properties, persistence, hazard and uses of the substance were received.

Although this substance 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. Therefore, this assessment focuses principally on information relevant to the evaluation of ecological risks.

Screening assessments focus on information critical to determining whether a substance meets the criteria as set out in section 64 of CEPA 1999[1]. Screening assessments examine scientific information and develop conclusions by incorporating a weight-of-evidence approach and precaution.

This final screening assessment includes consideration of information on chemical properties, hazards, uses and exposure, including the additional information submitted under the Challenge. Data relevant to the screening assessment of this substance were identified in original literature, review and assessment documents, stakeholder research reports and from recent literature searches, up to December 2009. Key studies were critically evaluated, along with modelling results, to reach conclusions.

When available and relevant, information presented in hazard assessments from other jurisdictions was considered. The final screening assessment does not represent an exhaustive or critical review of all available data. Rather, it presents the most critical studies and lines of evidence pertinent to the conclusion.

This final screening assessment was prepared by staff in the Existing Substances Programs at Health Canada and Environment Canada and incorporates input from other programs within these departments. The ecological portions of this assessment have also undergone external written peer review/consultation. Additionally, the draft of this screening assessment was subject to a 60-day public comment period. While external comments were taken into consideration, the final content and outcome of the screening assessment remain the responsibility of Health Canada and Environment Canada. Approaches used in the screening assessments under the Challenge have been reviewed by an independent Challenge Advisory Panel.

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

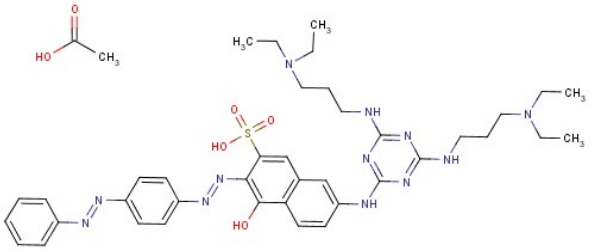
[1] A determination of whether one or more of the criteria of section 64 are met is based upon an assessment of potential risks to the environment and/or to human health associated with exposures in the general environment. For humans, this includes, but is not limited to, exposures from ambient and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

Substance Identity

Substance Name

For the purposes of this document, this substance will be referred to as NDTHPM, derived from the Domestic Substances List (DSL) name, [2-naphthalenesulfonic acid, 7-[[4,6-bis[[3-(diethylamino)propyl]amino]-1,3,5-triazin-2-yl]amino]-4-hydroxy-3-[[4-(phenylazo)phenyl]azo]-, monoacetate (salt)]. This synthetic organic dye has not been ascribed a Colour Index name (C.I.; CII 2002–) or a commercial name.

Table 1. Substance identity for NDTHPM

Chemical Abstracts Service Registry Number (CAS RN)	**71032-95-6**
DSL name	**2-Naphthalenesulfonic acid, 7-[[4,6-bis[[3-(diethylamino)propyl]amino]-1,3,5-triazin-2-yl]amino]-4-hydroxy-3-[[4-(phenylazo)phenyl]azo]-, monoacetate (salt)**
National Chemical Inventories (NCI) names[1]	*2-Naphthalenesulfonic acid, 7-[[4,6-bis[[3-(diethylamino)propyl]amino]-1,3,5-triazin-2-yl]amino]-4-hydroxy-3-[[4-(phenylazo)phenyl]azo]-, monoacetate (salt)* (AICS, ASIA-PAC, ENCS, PICCS, TSCA) *7-[[4,6-bis[[3-(diethylamino)propyl]amino]-1,3,5-triazin-2-yl]amino]-4-hydroxy-3-[[p-(phenylazo)phenyl]azo]naphthalene-2-sulphonic acid, monoacetate* (EINECS)
Other names	
Chemical group (DSL Stream)	Organics
Major chemical class or use	Amphoteric azo dyes
Major chemical sub-class	Cationic direct dyes; acetic and sulfonic acids
Chemical formula	$C_{39}H_{50}N_{12}O_4S.C_2H_4O_2$
Chemical structure	
SMILES[2]	O=S(=O)(c3cc2c(c(c3N=Nc4ccc(cc4)N=Nc5ccccc5)O)ccc(c2)Nc1nc(nc(n1)NCCCN(CC)CC)NCCCN(H)(OC(=O)C)(CC)CC)O
Molecular mass	843.02 g/mol

[1] National Chemical Inventories (NCI). 2007: AICS (Australian Inventory of Chemical Substances); ASIA-PAC (Asia-Pacific Substances Lists); EINECS (European Inventory of Existing Commercial Chemical Substances); ENCS (Japanese Existing and New Chemical Substances); PICCS (Philippine Inventory of Chemicals and Chemical Substances); and TSCA (Toxic Substances Control Act Chemical Substance Inventory).

[2] Simplified Molecular Input Line Entry System

Physical and Chemical Properties

NDTHPM is a cationic azo dye. It is also classified as an amphoteric dye, as the molecule can either donate or accept a proton, thus acting as an acid or base, respectively. In water, the substance will be ionized throughout the environmentally relevant range of pH (see Environmental Fate section). In addition to chemical structure, dyes may be classified according to their industrial applications and the methods by which they are applied to the substrate of interest (ETAD 1995; Hunger 2003). NDTHPM is a direct dye, which has extremely high affinity to bleached and wood-containing paper fibres due to its cationic charge and substantive properties (i.e., high degree of adsorption), and therefore it does not require any additional fixatives (Hunger 2003). Cationic direct dyes only retain their substantive character when the cationic charge is independent of the chromophore and not present in the chromophore in a delocalized form (Hunger 2003).

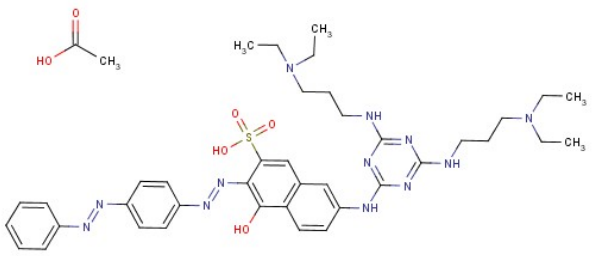
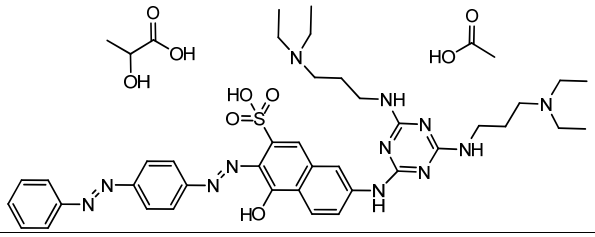
Few experimental data are available on the physical and chemical properties of NDTHPM. At the Environment Canada-sponsored Quantitative Structure-Activity Relationship (QSAR) Workshop in 1999, Environment Canada and other invited modelling experts identified many structural classes of pigments and dyes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

Environment Canada considers that when QSAR models are used to predict the physical and chemical properties of substances like NDTHPM, results may be quite uncertain. Consequently, a read-across approach has been used whenever possible to determine the approximate physical and chemical properties of a substance. A read-across approach has been used for NDTHPM based on an analogue with available experimental data (including physical/chemical and aquatic toxicity data). A search for suitable analogues was performed using the OECD's (Q)SARs Application Toolbox (QSARs 2008) and ChemIDplus Advanced (2005–) databases. The substance, Basic Red 111 (CAS RN 118658-98-3; C.I. 284240, CII 2002–), was chosen as a suitable structural analogue of NDTHPM for the purposes of this assessment. Basic Red 111 is a disazo dye with colouring in the bluish red shade for use on paper (CII 2002–).

Both Basic Red 111 and NDTHPM possess the same sulfonic acid core structure, 7-[4-(3-diethylaminopropylamino)-6-(3-diethylammoniopropylamino)-1,3,5-triazin-2-ylamino]-4-hydroxy-3-(4-phenylazophenylazo)-naphthalene-2-sulfonate (CAS RN 71032-94-5). The difference between the two substances is that NDTHPM is the acetate salt while Basic Red 111 is the acetate/lactate salt. The sulfonic acid group, as well as the lactic acid group in the analogue, contribute to high water solubility. These substances have similar molecular weights and similar cross-sectional diameters (they are relatively large

molecules), and are thus anticipated to behave similarly in the environment and demonstrate similar toxicities in the aquatic environment as a function of bioavailability and chemical reactivity. Structural information for these substances is presented in Table 2a below. See the Environmental Fate section for a discussion on how their ionic natures affect their behaviours in the environment.

Table 2a. Structural characterization of NDTHPM and Basic Red 111

CAS RN	Molecular Mass	Chemical Structure	Min-Max Maximum Diameter[1] (D_{max}) nm
71032-95-6 (NDTHPM)	843.02		2.6–3.4
118658-98-3 (Basic Red 111)	843.02-861.04		2.6–3.4

[1] Source: CPOPs 2008

Table 2b contains experimental and modelled physical and chemical properties of NDTHPM and Basic Red 111 that are relevant to their environmental fate. Key studies reporting experimental data (i.e., water solubility and partition coefficient for Basic Red 111) were critically evaluated and these reviews (as robust study summaries) are found in Appendices 1 and 2.

Table 2b. Physical and chemical properties for NDTHPM and the analogue, Basic Red 111

Chemical	Type	Value	Temperature (°C)	Reference
Physical state				
Basic Red 111	Experimental	Brown solid	25	Study Submission 2009a, 2009b
Density (kg/m^3)				
	No information available			
Vapour pressure (Pa)				
NDTHPM	Modelled[a]	2.0×10^{-35} (1.5×10^{-37} mmHg)	25	MPBPVP 2008
Henry's Law constant ($Pa \cdot m^3/mol$)				
NDTHPM	Modelled[a]	3.3×10^{-31} (3.3×10^{-36} $atm \cdot m^3/mole$)		HENRYWIN 2008
Log K_{ow} (Octanol-water partition coefficient) (dimensionless)				
Basic Red 111	Experimental[b,c]	< -0.33	20 – 25	Study Submission 2009a
Log D (Distribution coefficient) (dimensionless)[d]				
NDTHPM	Modelled (Log D_{ow}; octanol-water)	2.2 – 2.5	pH 6 – 9	ACD/PhysChem Suite 2009
	Modelled (Log D_{oc}; organic carbon-water)	~ 1	pH 6 – 9	ACD/PhysChem Suite 2009
Water solubility (mg/L)				
NDTHPM	Modelled	430, 84, 9.4, 1	pH 6, 7, 8, 9	ACD/PhysChem Suite 2009
Basic Red 111	Experimental[b]	> 3.4×10^5	25	Study Submission 2009b
Other solubilities (mg/L)				
NDTHPM	Experimental	Soluble in alcohol and acetone		Study Submission 2006
Basic Red 111	Experimental	1.6×10^5 (n-octanol)	25	Study Submission 2009a

Values in parentheses represent the original ones as reported by the authors or as estimated by the models.

[a]Input to models included SMILES notation in Table 1, as well as water solubility and log K_{ow} for the analogue Basic Red 111 (as shown above).

[b]Properties used for further modelling in the assessment.

[c]The partition coefficient was determined as a preliminary estimate using the log of the quotient of the n-octanol and water solubility of the test substance because it showed a clear surface active behaviour; as a result, OECD Guidelines 117 and 107 could not be applied (according to the authors). The purity of the substance was reported as 72% and the pH was not reported.

[d] The distribution coefficient takes into account the presence of the ionic species; represents the net amount of the neutral and ionic forms expected to partition into the lipid or organic carbon phases at a given pH. Log D_{ow} values at environmentally relevant pH given.

Since most of these QSAR models only accept the neutral form of a chemical as input (in SMILES form), most of the modelled values shown in Table 2b are for the neutral form of NDTHPM. These models are based on fragment addition methods, meaning they rely on the structure of the chemical. The SMILES notation shown in Table 1 (which includes the counterion) and the water solubility and log K_{ow} for the analogue were used as input to the model. Vapour pressure and Henry's Law constant have been predicted for NDTHPM due to the lack of experimental data. The ACD/PhysChem Suite (2009) can model some properties in relation to pH. Log D, which is the predicted distribution coefficient, taking into account the presence of the ionic species, represents a net amount of the neutral and ionic forms expected to partition into the lipid or organic carbon phases at a given pH. Modelled log D values at environmentally relevant pH are included in Table 2b.

The experimental water solubility (~340 g/L) and measured log K_{ow} (-0.33) for Basic Red 111 were used for further modelling in this assessment. Experimental data is preferable for this purpose. In comparison, it appears that the predicted log K_{ow} for NDTHPM (6.56; KOWWIN 2008), based on fragment addition methods and not considering the ionizing potential of the substance, would significantly over-estimate the actual net partitioning of the charged forms of the substance. Due to the inherent uncertainties associated with predicted values, expert judgment has been used in the interpretation of such data in this assessment.

Sources

NDTHPM does not naturally occur in the environment.

No reports of manufacture in or import into Canada of NDTHPM at or above the reporting threshold of 100 kg/year were received in response to an industry survey conducted for the year 2005 (Canada 2006b; Environment Canada 2006a). Therefore, NDTHPM was not initially included in the Challenge. NDTHPM was added to the Challenge as a result of information received during public comments on the draft screening assessment report on 148 persistent, bioaccumulative and inherently toxic (PBiT) substances that were believed to be not in commerce in Canada in 2005 (Canada 2006c) along with the Notice of Intent to apply the Significant New Activity provisions to these substances. The information submitted indicated that a total of between 100 and 1000 kg of this substance was imported into Canada in 2005 above the reporting threshold of 100 kg/year (Environment Canada 2006b).

Information was collected through an industry survey conducted for the year 2006 under a *Canada Gazette* notice issued pursuant to section 71 of CEPA 1999 (Canada 2009b). This notice required submission of data on the Canadian manufacture and import of

NDTHPM. Data were also required on use quantities of this substance. In association with the section 71 notice for 2006, companies that did not meet the mandatory reporting requirements but that had an interest in this substance were invited to identify themselves as stakeholders.

No companies reported manufacturing NDTHPM above the 100 kg/year threshold in 2006 (Environment Canada 2009a). Fewer than four companies imported a total of between 100 and 1000 kg of this substance into Canada above the 100 kg/year reporting threshold. No companies reported using this substance above the 1000 kg/year threshold and no companies reported a stakeholder interest.

During the DSL nomination, a total of between 100 kg and 1000 kg of NDTHPM were reported to be manufactured, imported or in commerce in Canada during 1986 (Environment Canada 1988).

The national aggregate production quantity for NDTHPM in the United States was 4500–227 000 kg in each of the 1990, 1994, 1998 and 2002 reporting cycles under the U.S. Environmental Protection Agency's Inventory Update Reporting program (US EPA 1986–2002). NDTHPM was also used in Sweden from 1999 to 2007 and in Finland from 2001 to 2002 (SPIN 2008). NDTHPM is in the European Inventory of Existing Commercial Chemical Substances (EINECS) but has not been reported as either a high production or low production volume chemical (ESIS c1995–2009).

Uses

Information submitted for 2006 as a result of the section 71 survey was identified as Confidential Business Information (CBI; Environment Canada 2009a).

In Canada, NDTHPM is not approved for any food additive use nor has Health Canada received submissions for its use in food packaging materials or formulations of incidental additives (2010 personal communication from Foods Directorate, Health Canada, to Existing Substances Risk Assessment Bureau, Health Canada; unreferenced).

During the DSL nomination (1984–1986), the DSL use code for "Colourant - pigment/stain/dye/ink" was identified for NDTHPM (Environment Canada 1988).

NDTHPM is a cationic direct dye used for colouring paper with red hues (Hunger 2003). It is recommended for all grades of tissue, fine paper and paperboard under both acid and alkaline conditions (Study Submission 2009c). Cationic direct dyes have extremely high affinity to bleached and wood-containing paper fibres due to their cationic charge and substantive properties (Hunger 2003). In addition, the dye has a very good fastness property, with trace to very slight bleed in water (Study Submission 2009c). The Substances in Preparations in Nordic Countries database also indicates that NDTHPM was used as a colouring agent for pulp and paper products in Finland (SPIN 2008).

Releases to the Environment

NDTHPM is not reportable to the National Pollutant Release Inventory (NPRI 2007) or to the US Toxics Release Inventory Program (TRI 2006).

A method has been developed by Environment Canada to estimate a substance's losses during different stages of its life cycle, including its fate within a finished product or article (Environment Canada 2008). This method consists of a life cycle analysis and a spreadsheet tool (Mass Flow Tool or MFT) that integrates information on the manufacturing, importation and use data available for the substance. Starting with an identified mass of the substance, each life cycle stage is subsequently evaluated until all of the mass is accounted for. Relevant factors are considered, uncertainties recognized and assumptions may be made during each stage, depending on information available. The estimated losses represent the complete mass balance of the substance over the life cycle of the substance and include releases to wastewater and other receiving compartments (land, air), chemical transformation, transfer to recycling activities and transfer to waste disposal sites (landfill, incineration). However, unless specific information on the rate or potential for release of the substance from landfills and incinerators is available, the method does not quantitatively account for releases to the environment from disposal. Ultimately, the estimated losses provide a first tier in the exposure analysis of a substance and help to estimate environmental releases and focus exposure characterization later in the assessment.

In general, releases of a substance to the environment depend upon various losses from its manufacture, industrial use and consumer/commercial use. These losses can be grouped into seven types: (1) discharge to wastewater; (2) emission to air; (3) loss to land; (4) chemical transformation; (5) disposal to landfill; (6) loss to incineration; and (7) disposal through recycling (i.e., recycling is deemed a loss and not considered further). They are estimated using regulatory survey data, industry data and data published by different organizations. The discharge to wastewater refers to raw wastewater prior to any treatment by public or private wastewater systems. In a similar manner, the loss via chemical transformation refers to changes in a substance's identity that may occur within the manufacture, industrial use, and consumer/commercial use stages, but excludes those during waste management operations such as incineration and wastewater treatment. The loss to land includes unintentional transfer or leakage to soil or paved/unpaved surfaces during the substance's use and service life (e.g., from the use of agricultural machinery or automobiles). The loss to land, however, does not include transfers subsequent to a substance's use and service life (e.g., land application of biosolids and atmospheric deposition).

The losses estimated for NDTHPM over its life cycle (based on conservative assumptions) are presented in Table 3 (Environment Canada 2009b). NDTHPM is not

manufactured in Canada above reporting thresholds, so estimated losses are based on import quantities reported in 2006.

Table 3. Estimated losses of NDTHPM during its life cycle using the MFT

Type of loss	Proportion (%)	Pertinent life cycle stages
Wastewater	10.5	Industrial use
Land	0	
Air emission	0	
Chemical transformation	0	
Incineration	1.2	Consumer/commercial use
Landfill	36.4	Consumer/commercial use
Recycling	51.9	Consumer/commercial use
Total	100	

NDTHPM is estimated to be released in industrial wastewater at about 11% during the industrial use stage. Assumptions made during this step include losses during container handling and dyeing operations. The majority of NDTHPM is estimated to be lost through waste disposal (incineration ~1% and landfill ~36%) and recycling to recycled paper plants (~52%). No releases from paper products were assumed during consumer/commercial use of the substance, given that paper is generally kept protected from water. Cationic direct dyes used as paper dyes have a very high substantivity (degree of adsorption) and very high affinity (degree of fixation) to the substrate (ETAD 1992), and when added to paper during processing operations, these types of dyes become chemically incorporated into the matrix of the paper thereby reducing the potential for release into the environment during product use or disposal (Hunger 2003; Study Submission 2009c). Finally, losses to recycling activities were estimated based on available information on the paper recycling rate (~58%) by Canadian industries.

The above loss estimates indicate that NDTHPM has a potential for release to the environment. With respect to wastewater treatment, most of the adsorption/desorption research on dyes has been done using activated sewage sludge or carbon (ETAD 1995), with dyestuffs generally being adsorbed to the extent of 40–80% (Clarke and Anliker 1980). Therefore, there is the potential for NDTHPM to be released to water and/or to be applied to soil through wastewater treatment plant biosolids used as soil enrichment. Additional releases to water and soil are also possible as a result of recycling activities.

Environmental Fate

Based on its physical and chemical properties (Tables 2a and 2b), use as a paper dye, and the MFT loss estimates, NDTHPM is expected to be found mainly in water and/or soil as a result of releases to the environment.

If released to the aquatic environment, NDTHPM is expected to be found mostly in the water column, as the substance is anticipated to be very water soluble due to the read-

across data available for the analogue Basic Red 111 (~3.4 x 10^5 mg/L). NDTHPM ionizes completely in water (Environment Canada 2009c). The acetic acid group will dissociate from the core sulfonic acid structure in water at ambient pH. The remaining sulfonic acid core structure will be ionized, with six forms possible in water, each with a different net charge depending on the pH. Between pH 6 and 9, the sulfonic acid group will donate a proton (and be negatively charged) and the two tertiary amines will accept protons (and be positively charged), giving the molecule a net positive charge. This form of the molecule is the main one present (comprising 91–99% of the molar mass in water) within this pH range. At pH 5, approximately 28% of the molecules have a net charge of +2, having an additional positive charge on the triazine group. Therefore, both negative and positive charges are present in the molecule at environmentally relevant pH, hence its amphoteric characterization.

In addition, NDTHPM's adsorption characteristics would be influenced by these ionic groups. This substance would have a relatively high binding affinity to both negatively and positively charged particles in the water column, and thus would be expected to adsorb to suspended solids and eventually settle in bed sediments or wastewater sludge. Razo-Flores et al. (1997) suggest that due to the recalcitrant nature of azo dyes in aerobic environments, they eventually end up in anaerobic sediments, shallow aquifers and groundwater.

If released to soil, the ionic nature of this dye may result in binding through cation-exchange processes with clay minerals or with solid organic matter that would retard leaching.

Given the intended use in aqueous-based treatments, NDTHPM is not expected to be released to air and is not expected to partition to this compartment, based on a very low modelled vapour pressure (2.0 x 10^{-35} Pa) and a negligible Henry's Law constant (3.3 x 10^{-31} Pa·m^3/mol). As the cationic form of this substance dominates at environmentally relevant pH, volatilization will be negligible.

Persistence and Bioaccumulation Potential

Environmental Persistence

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

Biotic degradation of azo dyes may take place relatively rapidly under anaerobic or reducing conditions (Baughman and Weber 1994; Danish EPA 1999; ETAD 1995; Isik and Sponza 2004; Yen et al. 1991). Permeability through the bacterial cell wall has been found to be the rate-limiting step in the reduction process (Danish EPA 1999). Under anaerobic conditions, azo dyes have a high tendency to cleave at the azo bond with the formation of aromatic amines (Danish EPA 1999; Hunger 2005). The carcinogenic potential of aromatic amines varies considerably (see Health Effects section). However, the formation of such metabolites in deep anoxic sediments would typically not result in exposure to aquatic organisms.

There are limited experimental data on the persistence of NDTHPM in the environment. The studies available are inherent biodegradation tests (evaluating primary biodegradation) (Study Submission 2006, 2009d), which generally provide favourable conditions for biodegradation compared with ready biodegradation tests. The results, however, are inconclusive and seem to indicate that some loss mechanisms may be involved other than biodegradation.

One study evaluated the inherent biodegradation of NDTHPM by wastewater bacteria (without pre-adaptation) using a modified MITI 28-day test (Study Submission 2006). Biological oxygen demand (BOD) was measured and biodegradation was estimated using the ratio of BOD to the chemical or theoretical oxygen demand when the compound is completely oxidized. The extent of biodegradation ranged between 125 and 226% after 28 days. Some degradation was also observed in the flask without inoculum (20%) after 28 days. These results are uncertain, particularly given the reported biodegradation values in excess of 100%.

In another study, inherent biodegradation was investigated for Basic Red 111 (72% purity) using a modified Zahn-Wellens test and micro-organisms from a domestic wastewater sewage plant (Study Submission 2009d). Results showed that the test substance was not completely dissolved at the beginning of the test, and after seven days, strong foaming of the test solutions caused some material to be deposited on the covers of the vessels. Dissolved organic carbon values decreased by 87% after the strong foaming observed on day seven of exposure. Therefore, it was not clear whether the test substance was biodegraded or adsorbed to the glass covers.

Because of the overall uncertainty in these experimental data, and also given that inherent biodegradation tests are generally less stringent and less preferable to ready biodegradation tests, a QSAR-based weight-of-evidence approach (Environment Canada 2007) was also applied using the degradation models shown in Table 4 below. These models are considered acceptable for use as they are based on chemical structure, and the disazo structure is represented in the training sets of all the BIOWIN models used, thereby increasing the reliability of the predictions. Given the ecological importance of the water compartment, the fact that most of the available models apply to water and the fact that NDTHPM is expected to be released to this compartment, persistence in water was primarily examined. NDTHPM does not contain functional groups expected to undergo hydrolysis.

Table 4. Modelled data for biodegradation of NDTHPM

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
WATER			
Primary biodegradation			
Biodegradation (aerobic)	BIOWIN 2008[a] Sub-model 4: Expert Survey (qualitative results)	1.92[b] "biodegrades slowly"	≥ 182
Ultimate biodegradation			
Biodegradation (aerobic)	BIOWIN 2008[a] Sub-model 3: Expert Survey (qualitative results)	0.05[b] "biodegrades very slowly"	≥ 182
Biodegradation (aerobic)	BIOWIN 2008[a] Sub-model 5: MITI linear probability	-1.65[c] "biodegrades very slowly"	≥ 182
Biodegradation (aerobic)	BIOWIN 2008[a] Sub-model 6: MITI non-linear probability	0.00[c] "biodegrades very slowly"	≥ 182
Biodegradation (aerobic)	TOPKAT 2004 Probability	0.0[c] "biodegrades very slowly"	≥ 182
Biodegradation (aerobic)	CATABOL c2004–2008 % BOD	% BOD = 0.3 "biodegrades very slowly"	≥ 182

[a] EPI Suite (2008), using SMILES notation in Table 1.

[b] Output is a numerical score from 0 to 5.

[c] Output is a probability score.

The results in Table 4 for the aerobic biodegradation models (BIOWIN 3, 4, 5, 6, TOPKAT, and CATABOL) all indicate that NDTHPM biodegrades to mineral constituents very slowly with a half-life that is likely to be greater than 182 days. This finding is consistent with what would be expected for this chemical structure (i.e., few degradable functional groups) and its intended use as a dye.

Using an extrapolation ratio of 1:1:4 for a water: soil: sediment biodegradation half-life (Boethling et al. 1995) and using the model-estimated ultimate biodegradation half-life in water of ≥ 182 days, the ultimate degradation half-life in aerobic soil is also expected to be ≥ 182 days and the half-life in aerobic sediment is expected to be ≥ 365 days. As mentioned previously, however, experimental evidence for some azo dyes suggests that this dye may not persist in the deeper, anoxic layers of sediments where azo bonds are readily reduced under anaerobic conditions.

Based on the modelled data (Table 4), it is concluded that NDTHPM meets the persistence criteria in water, soil and sediment (half-lives in soil and water ≥ 182 days and half-life in sediment ≥ 365 days), as set out in the *Persistence and Bioaccumulation Regulations* (Canada 2000).

Potential for Bioaccumulation

In this assessment, a variety of lines of evidence have been used to determine the bioaccumulation potential of NDTHPM, which is different from the approach used during categorization—that relied on outputs from QSARs using the predicted log K_{ow} of the neutral form of the substance. As indicated in Table 2b, this substance is expected to be highly water soluble based on the read-across data for Basic Red 111 (~340 g/L). In addition, the experimental log K_{ow} value of -0.33 for Basic Red 111 indicates that NDTHPM would have a low bioaccumulation potential.

Estimated and experimental log K_{ow} values were compared with experimental bioconcentration factors (BCFs) for fish for a number of dyes (Anliker et al. 1981; Danish EPA 1999; ETAD 1995). With respect to the data for six acid dyes and one direct dye (only dye classes were reported), reported BCFs were less than 10, indicating that these very hydrophilic (ionic) dyes are not likely to bioconcentrate in aquatic organisms.

There are no empirical bioaccumulation data available for NDTHPM. Therefore, available data on water solubility, molecular weight, cross-sectional diameter, and modelled BCF and bioaccumulation factors (BAFs) have been considered in order to determine the bioaccumulation potential of this substance.

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

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

NDTHPM is a relatively large molecule with a high molecular weight (843 g/mol), a D_{eff} range from 1.2–2.0 nm (CPOPs 2008), and a D_{max} range from 2.6–3.4 nm (CPOPs 2008), which are comparable to some of the values cited above. These characteristics suggest that the uptake rate of this substance may be slower compared to that of smaller more compact substances, thus mitigating the overall bioconcentration potential.

No experimental BAF and/or BCF data for NDTHPM were available, so a predictive approach was applied using available BAF and BCF models as shown in Table 5. According to the *Persistence and Bioaccumulation Regulations* (Canada 2000), a substance is bioaccumulative if its BCF or BAF are ≥ 5000. All estimated BCFs and the BAF are much lower than the 5000 threshold.

Table 5. Modelled data for bioaccumulation

Test organism	Endpoint	Value wet weight (L/kg)	Reference
Fish	BCF[1] (neutral form)	0.94 (with metabolism)	Arnot and Gobas 2003 (Arnot-Gobas middle trophic level)
Fish	BAF[1] (neutral form)	0.94 (with metabolism)	
Fish	BCF[1] (corrected for metabolism)	7.65	CPOPs 2008
Fish	BCF[1] (accounts for ionization potential)	3.16	BCFBAF 2008

[1] The log K_{ow} for the analogue Basic Red 111 (-0.33) and the SMILES notation in Table 1 were used for model predictions using EPI Suite (2008).

BCF and BAF estimates, corrected for potential whole body biotransformation, were generated using the BCFBAF model (EPIsuite 2008). The middle trophic level fish in the Arnot-Gobas model was used to represent overall model output, as suggested by the model developer, as it is most representative of fish weight likely to be consumed by an avian or terrestrial piscivore. The model outcomes indicate that NDTHPM has a low potential to bioaccumulate in fish. As discussed previously, NDTHPM is also expected to have a low bioaccumulation potential due to its physical and chemical properties (i.e., high molecular weight, steric effects, charged nature). The physical and chemical properties of the relevant analogue, Basic Red 111, particularly its measured high water solubility and low experimental log K_{ow} also support this evaluation.

Therefore, considering the available evidence, it is concluded that NDTHPM does not meet the bioaccumulation criteria (BCF or 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

A variety of lines of evidence have been used to determine the toxicological potential of NDTHPM, which is different from the approach used during categorization that relied mainly on log K_{ow}-based QSAR predictions. The available data to support this assessment are discussed below.

The available empirical data for NDTHPM and the analogue Basic Red 111 are summarized in Table 6a.

Table 6a. Empirical data for aquatic toxicity

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
NDTHPM				
Rainbow Trout (*Oncorhynchus mykiss*)	Acute 96-hour	LC_{50}	> 10 and < 100	Study Submission 2006
Wastewater bacteria (spp.)	Respiratory inhibition test	IC_{50}	> 100	
Basic Red 111				
Zebra Fish (*Danio rerio*)	Acute 24-hour	LC_{50}	31	Study Submission 2009e
	Acute 96-hour	LC_{50}	16.7	
		NOEC	10	
Daphnia magna	Acute 24-hour	EC_{50}	194	Study Submission 2009f
	Acute 48-hour	EC_{50}	109	
		NOEC	32	
Rainbow Trout (*Oncorhynchus mykiss*)	Chronic 21-day	LC_{50}	13	Study Submission 2009g
		LOEC	6*	
		NOEC	2.7	

EC_{50} – The concentration of a substance that is estimated to cause some effect on 50% of the test organisms.
IC_{50} – The inhibiting concentration for a specified percent effect. A point estimate of the concentration of a test substance that causes 50% reduction in a quantitative biological measurement such as growth rate.
LC_{50} – The concentration of a substance that is estimated to be lethal to 50% of the test organisms.
LOEC – The lowest-observed-effect-concentration is the lowest concentration in a toxicity test that caused a statistically significant effect in comparison to the controls.
NOEC – The no-observed-effect concentration is the highest concentration in a toxicity test not causing a statistically significant effect in comparison to the controls.
* Value used to calculate the predicted no-effects concentration.

The empirical study for NDTHPM assessed the toxicity of this substance (mixed with Tween 80) on Rainbow Trout over a 96-hour period in a static system (Study Submission 2006). The test (nominal) concentrations were 0.1, 1.0, 10.0 and 100.0 mg/L. There were 10 fish (average weight of 1 g) per treatment. No mortality was observed in the control with Tween 80, at 0.1 mg/L or 1.0 mg/L, while 10% mortality (one fish) was observed at

10.0 mg/L. All test organisms died at the highest concentration tested (100.0 mg/L). The study was deemed acceptable for consideration in this assessment, and the corresponding robust study summary is attached in Appendix 3.

Experimental data on NDTHPM and Basic Red 111 (considering similar endpoints) agree within approximately one order of magnitude. It would be expected that they would have similar toxicities, given their structural similarities. These data suggest that NDTHPM exhibits moderate (bordering on high) to low acute toxicity to aquatic organisms (i.e., LC_{50}s and EC_{50}s 1 mg/L to > 100 mg/L), depending on species selected. A LOEC value of 6 mg/L for Basic Red 111 was also reported from a chronic study on Rainbow Trout (Study Submission 2009g, see the robust study summary in Appendix 4). This value was used to derive the predicted no-effects concentration (PNEC) as described later in this report.

As mentioned previously, cleavage of the azo bond under anaerobic or reducing conditions (e.g., deep layers of sediments) is known to result in aromatic amines, some of which are known to be potentially harmful. However, because they are only formed in deep anoxic sediment, there is little likelihood that aquatic organisms are exposed to these more harmful metabolites.

Experience with over 200 acid dyes has led to the observation that the potential ecotoxicity of such substances may generally be predicted by the number of acid groups present (US EPA 2002). Both NDTHPM and Basic Red 111 contain one sulfonic acid group. Some monoacid and diacid dyes have shown high to moderate toxicity (i.e., acute values < 1 mg/L and < 100 mg/L, respectively) to fish and other aquatic organisms. Dyes with three or more acid groups showed low toxicity (i.e., acute values > 100 mg/L) towards fish and invertebrates. All acid dyes showed moderate toxicity to green algae, with further analysis suggesting that such effects may have been related to shading. For these generalizations to be applicable, the acid dyes must have some water solubility and molecular weights generally need to be near or below 1000, which is the case for both NDTHPM and Basic Red 111. In addition, the tertiary amines contained in the molecule of NDTHPM could be contributing to its toxicity.

In evaluating the aquatic toxicity of NDTHPM, modelled data using the QSAR weight-of-evidence approach (Environment Canada 2007) have also been used to predict the potential aquatic toxicity of this substance, recognizing the uncertainties associated with using these models for ionizing substances (Table 6b). The log K_{ow} for the analogue Basic Red 111 was used in making these calculations, unless otherwise indicated.

Table 6b. Modelled data for aquatic toxicity

Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Fish	Acute (96 hours)	LC_{50}	3.7×10^4	ECOSAR 2008 (Aliphatic amines)
Fathead Minnow *Pimephales promelas*	Acute (96 hours)	LC_{50}	311	AIEPS 2003–2007
Fathead Minnow *Pimephales promelas*	Acute (96 hours)	LC_{50}	≤ 2.3	CPOPs 2008
Daphnid	Acute (48 hours)	LC_{50}	2328	ECOSAR 2008 (Aliphatic amines)
Daphnia magna	Acute (48 hours)	LC_{50}	11.0	AIEPS 2003–2007
Daphnia magna	Acute (48 hours)	EC_{50}	≤ 1.8	CPOPs 2008
Algae	Acute (96 hours)	EC_{50}	469	ECOSAR 2008 (Aliphatic amines)
Algae *Pseudokirchneriella subcapitata*	Acute (72 hours)	EC_{50}	1.5	AIEPS 2003–2007

AIEPS (2003–2007): aqueous water solubility predicted by model = 12 337 mg/L; model would not allow user to define water solubility or log K_{ow} values
CPOPs (2008): model recognizes that reactive groups exist; result may be more toxic than value predicted; log K_{ow} of 0.33 used in model, based on data for the analogue, Basic Red 111
ECOSAR (2008): water solubility of 340 g/L and log K_{ow} of -0.33 used in model, based on data for the analogue, Basic Red 111.
EC_{50} – The concentration of a substance that is estimated to cause some effect on 50% of the test organisms.
LC_{50} – The concentration of a substance that is estimated to be lethal to 50% of the test organisms.

A range of aquatic toxicity predictions were obtained from the various QSAR models considered. Although there are uncertainties associated with these modelled data, the majority of these results are within the range of 1.5–469 mg/L (with the exception of the ECOSAR results for fish and daphnids), which is similar to the range for empirical data. These empirical and model estimates indicate that NDTHPM exhibits moderate (bordering on high) to low toxicity to aquatic organisms (i.e., LC_{50}s and EC_{50}s 1 mg/L to > 100 mg/L), depending on species selected.

B – In Other Environmental Compartments

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

NDTHPM may adsorb to solid particles and end up in soil or sediment, as discussed previously (see Environmental Fate section). Therefore, it would be desirable to have

toxicity data for organisms that reside in these media. No suitable ecological effects studies were found for the chemical in media other than water.

Ecological Exposure Assessment

No data concerning concentrations of NDTHPM in water in Canada have been identified; therefore, environmental concentrations are estimated from available information, including estimated substance quantities in commerce, release rates, and size of receiving water bodies.

A – Industrial Release

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

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

where

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

As NDTHPM is used industrially and is expected to be released to water, a worst-case industrial release scenario is used to estimate the aquatic concentration of the substance with the help of Environment Canada's (2009d) Industrial Generic Exposure Tool – Aquatic (IGETA). The scenario is made conservative by assuming that the total quantity of the substance used by Canadian industry is used by one single industrial facility at a small, hypothetical site and the loss to a local wastewater treatment plant is high at 10% of the total quantity resulting from the cleaning of chemical containers and processing equipment. Such a small site is selected to have an effluent flow at the 10^{th} percentile (3 456 m^3 /d) of the wastewater treatment plant discharge rates across Canada. The scenario also assumes that the release occurs 250 days per year (typical for small and medium-sized facilities), the wastewater treatment plant removal rate is zero for the substance, and the receiving water dilution factor is ten. Based on the above assumptions, a total industrial use quantity of 1000 kg/yr of the substance (the upper limit of the reporting range) yields a PEC in water of 0.01 mg/L (Environment Canada 2009e).

B – Consumer Release

NDTHPM is primarily used as a dye for paper and has a very high fixation rate, thereby reducing the potential for leaching. Regardless, releases of the dye from paper during consumer use are not expected, as most uses of paper are generally protected from water. Therefore, a consumer release scenario was not developed.

Characterization of Ecological Risk

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

NDTHPM is expected to be persistent in water, soil and sediment. It is expected to have a low bioaccumulation potential. Although importation quantities into Canada are relatively low, there is the potential for this substance to be released into the Canadian environment (see results of industrial release scenario). Once released into the environment, it will be found in water, but may eventually settle to bed sediments or will end up in wastewater treatment plant sludge. The available toxicity data indicate that it is likely to exhibit moderate to low toxicity to aquatic organisms.

A risk quotient analysis, integrating conservative estimates of exposure with toxicity information, was performed for the aquatic medium to determine whether there is potential for ecological harm in Canada. The industrial release scenario presented above yielded a PEC of 0.01 mg/L (Environment Canada 2009e). A PNEC was derived from the chronic toxicity value of 6 mg/L for Rainbow Trout (the most sensitive valid experimental value), by dividing this value by an assessment factor of 10 (to account for interspecies and intraspecies variability in sensitivity and extrapolation from lab to field) to give a value of 0.6 mg/L. The resulting risk quotient (PEC/PNEC) = 0.02.

This information suggests that NDTHPM has a low potential to cause ecological harm in Canada.

Uncertainties in Evaluation of Ecological Risk

In general, NDTHPM is a data-poor substance. The main uncertainty is associated with characterizing physical and chemical properties of NDTHPM. Due to the lack of information for NDTHPM on its physical and chemical properties, persistence, bioaccumulation potential, and toxicity to aquatic organisms, a read-across approach using data from a selected analogue was relied upon. Predictions using QSAR models

had to be used to fill in data gaps, although uncertainty exists with using such models to make predictions for ionizing substances in particular.

The persistence assessment is limited by the absence of reliable empirical biodegradation data, which necessitated the generation of model predictions. Although all model predictions have some degree of error, these model predictions are considered acceptable as they are based on chemical structure. The disazo structure is represented in the training sets of the models used, thereby increasing the reliability of the predictions. It is unclear to what extent NDTHPM would undergo anaerobic degradation in sediments to constitutive amines, although the potential amine degradation products are not expected to be biologically available because they would form only in relatively deep anoxic sediment.

The bioaccumulation assessment for NDTHPM was limited by the lack of empirical data. Bioaccumulation assessment relied on the interpretation of physical and chemical properties (especially log K_{ow}) for an analogue and models for predicting the bioaccumulation potential of NDTHPM.

The lack of information on environmental concentrations of this substance in Canada resulted in the need to evaluate risk based on predicted concentrations in water near an industrial point source. Conservative assumptions were made to estimate concentrations in receiving water bodies, which resulted in a relatively low predicted environmental concentration. Although releases of this substance into the Canadian environment are likely to be low because of the low quantity of the substance imported into Canada, it would be beneficial to have information on sediment or soil concentrations of this substance in Canada to ascertain the degree to which it accumulates in these media.

Aquatic toxicity information was also limited for this substance, although more data were available for its analogue. Additional chronic toxicity data on organisms other than fish would be beneficial in evaluating interspecies variability. The use of an assessment factor in determining a predicted no-effect concentration is intended to address this uncertainty along with the extrapolation from lab to field. 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. However, given the low quantity that is in use in Canada, exposure of organisms to soils or sediments containing this substance is not expected to be significant.

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

releases from waste disposal sites are possible, although difficult to quantify due to the lack of data, and would contribute to overall environmental concentrations.

Potential to Cause Harm to Human Health

Exposure Assessment

Environmental Media and Food

From the published literature, no data were identified on measured concentrations of NDTHPM in environmental media (air, water, soil and sediment) and food in Canada or elsewhere. In Canada, NDTHPM is not approved for any food additive use nor has the Food Directorate received submissions for its use in food packaging materials or formulations of incidental additives. Taken together, there is no expected exposure from food.

In the absence of release data from publicly available inventories or the section 71 survey of CEPA 1999 (Environment Canada 2009a), environmental releases were conservatively estimated using the loss percentages predicted by the MFT (see Table 3) applied to the maximum of the import quantity range reported in 2006 (1000 kg) (Environment Canada 2009b). Based on these estimated losses, ChemCAN, a Canadian-specific environmental exposure model (ChemCAN 2003), was used to predict concentrations in environmental media. The resulting conservative upper-bounding daily intakes of NDTHPM for the general population of Canada were in the order of nanograms per kg-bw (kilogram of body weight) per day.

The estimated upper-bounding daily intakes of NDTHPM for the general population of Canada are considered as the worst case scenario where some assumptions were very conservative. For example, the release quantity of NDTHPM to wastewater without treatment was used to estimate concentration of NDTHPM in surface water, which was then used as a surrogate for concentration of NDTHMP in drinking water. It was also considered that all NDTHPM leaches from landfill to soil. It is expected that the actual concentrations of NDTHPM in the environmental media is much lower than the estimated range of nanograms per kg-bw per day.

Consumer Products

NDTHPM is used as a dye for colouring paper with red hues (Hunger 2003). It may also be used as a colouring agent in other pulp and paper products (SPIN 2008). As an active dye component, it has very high fixation rates and does not leach. The maximum concentration of NDTHPM dye applied to paper products is 1% (2010 personal communication from Industry to Chemicals Sector Directorate, Environment Canada; unreferenced).

In Canada, paper products containing NDTHPM may be used for medical grade, offset, and copy paper. These paper products are expected to be sold to both industry and consumers (2010 personal communication from Industry to Chemicals Sector Directorate, Environment Canada; unreferenced).

Unlike inks, dyes are contained within a paper's matrix and would be expected to demonstrate negligible migration in a dermal scenario. However, children aged 0.5 to 4 years may have incidental exposure resulting from mouthing of paper leading to potential oral exposure. Despite a high fixation rate in mildly acidic salt baths, the stomach's highly acidic environment (pH ≈ 2) may affect dye fastness in an unknown manner. Therefore, it may be possible for this substance to become bioavailable in the gastrointestinal tract for absorption and/or azo-reduction by intestinal bacteria resulting in free aromatic amines (refer to Health Effects Assessment section for details).

Potential exposure to NDTHPM in paper dyes from incidental ingestion of paper by children was estimated (Appendix 5). The upper-bounding potential oral intake was estimated to be 0.6 mg/kg-bw per event for children aged 0.5 to 4 years who are likely to ingest paper by mouthing. The assumptions used to derive this estimate are very conservative. In addition, this type of exposure is considered incidental.

Inhalation and dermal exposures to NDTHPM from its presence in paper products were not estimated, as potential for exposure from these routes is considered to be negligible based on the current use of this substance, its physical and chemical properties, and high degree of fixation of the dye in paper.

Health Effects Assessment

No empirical hazard data were identified for NDTHPM. Therefore, information on potential metabolites, related salts, analogues of this substance, and properties associated with the broader chemical class has been considered.

It has been demonstrated that azo colorants can undergo reductive cleavage mediated by azo reductase 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). It is recognized that the degree of azo reduction is likely influenced by various factors (e.g., solubility of parent compound, presence and position of molecular substituents). Certain azo substances can cleave at the azo bond with the formation of aromatic amines (Danish EPA 1999; Hunger 2005). The carcinogenic potential of aromatic amines varies considerably with molecular structure, with carcinogenic breakdown products being associated with the moieties of benzidine, toluene or naphthalene for example (Danish EPA 1999; Hunger 2005).

A search for potential analogue substances in SciFinder identified 167 substances similar to NDTHPM (similarity ≥ 85%); however none of them were found to have relevant empirical health effects data.

Related salts (acetate-lactate salt, lactate salt) and the parent azo structure of NDTHPM were identified from searches on SciFinder (Appendix 7). While no empirical data were identified for these substances, the acetate-lactate salt, Basic Red 111 (CAS RN 118658-

98-3) has been included in a review of azo colorants by the European Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP 2002). The SCCNFP considered azo dyes, which may release one or more carcinogenic aromatic amines to pose a risk to consumers. Although focused on a number of specific azo colorants, the SCCNFP opinion applied more broadly to include other similar azo colorants, including Basic Red 111 for which a hazard was identified due to potential release of 4-aminoazobenzene (listed in Annex 3, Table 3, of SCCNFP 2002); one of the same predicted azo cleavage products of NDTHPM (see below). Basic Red 111 was also included on a list of azo dyes prohibited in India by the Indian Ministry of Environment and Forests) due to potential release of carcinogenic amines (Government of India 1997). The German Chemical Industry Association (VCI) has listed Basic Red 111 as potentially releasing 4-aminazobenzine by reductive cleavage and therefore a dye which should not be used for consumer goods (Dystar 2000, VCI 2001).

The potential azo cleavage products of NDTHPM are shown in Appendix 7 and relevant toxicity data have been identified for three of these: 4-aminoazobenzene (CAS RN 60-09-3), aniline (CAS RN 62-53-3), and *p*-phenylenediamine (PPD) (CAS RN 106-50-3). A brief summary of the toxicity data for these three potential azo cleavage products is presented below, with a focus on genotoxicity and carcinogenicity endpoints which are critical effects for the class of aromatic amines.

The potential azo cleavage product 4-aminoazobenzene (CAS RN 60-09-3) is a recognized animal carcinogen causing liver and skin tumours in rats, and was classified by IARC as Group 2B carcinogen (IARC 1975; 1987. In Europe, 4-aminoazobenzene is classified as a carcinogen under the European CLP Regulation (European Commission 2008, ESIS 2010) and is also included in REACH as one of the 22 aromatic amines of concern, which should not be released from azo colourants used in textiles and leather (REACH Annex XVII Appendix 8) (European Commission 2006). With regards to genotoxicity, 4-aminoazobenzene was predominantly positive for mutagenicity, clastogenicity and DNA damage in a number of genotoxicity assays both *in vivo* and *in vitro* (references cited in BfR 2003, IARC 1987, BIBRA 1989).

Aniline (CAS RN 62-53-3) has been previously evaluated by Health Canada and Environment Canada (Canada 1994; an update is currently in progress) and more recently by the European Union (EU 2004). Tumours of the spleen were observed following chronic dietary exposures in rats while no carcinogenicity was observed in mice. Genotoxicity studies for aniline were mixed overall, with positive and negative results reported for *in vivo* and *in vitro* assays.

PPD (*p*-phenylenediamine; CAS RN 106-50-3) was recently evaluated by the European Scientific Committee on Consumer Products (SCCP 2006). Multiple carcinogenicity studies were identified. A chronic feeding study in B6C3F1 mice and F344 rats did not show a substance-specific carcinogenic effect (SCCP 2006). *In vitro* genotoxicity data for PPD are mixed, with negative and positive results for mutagenicity and clastogenicity, while *in vivo* assays were negative for bone marrow clastogenicity, hepatocyte

unscheduled DNA synthesis (UDS), comet assay, and DNA binding. PPD is currently listed on Health Canada's Cosmetics Ingredient Hotlist (Health Canada 2009).

No chemical-specific toxicity or metabolism data were identified for NDTHPM. However, based on evidence of azo cleavage for the class of azo substances, it is considered that exposure to NDTHPM may potentially result in exposure to its corresponding azo cleavage products, in particular 4-aminoazobenzene.

The confidence in the health effects database for NDTHPM is considered to be low due to a lack of substance-specific data, however there is relatively robust hazard information on the potential azo cleavage products.

Characterization of Risk to Human Health

There were no empirical toxicity data available on NDTHPM to characterize the human health effects. However, health effects data on the potential azo cleavage products have been considered in the hazard characterization for human health. In particular, 4-aminoazobenzene has been classified as an IARC Group 2B carcinogen based on tumours in rats following oral and dermal exposure (IARC 1975, 1987) and has also demonstrated positive genotoxicity in a range of assays *in vivo* and *in vitro* (references cited in BfR 2003, BIBRA 1989). In the absence of chemical-specific data on NDTHPM, it is considered that 4-aminoazobenzene may be potentially released by cleavage of the azo bond. Similarly, the acetate-lactate salt of NDTHPM, Basic Red 111 has also been identified as a potential health concern based on the same potential release of 4-aminoazobenzene from azo cleavage (SCCNFP 2002) and has been banned for use in India (Government of India 1997). Therefore, on the basis of the potential release of 4-aminoazobenzene from cleavage of the azo bond, it is considered that there is a potential hazard associated with NDTHPM.

The potential for exposure of the general population to NDTHPM from environmental media is expected to be negligible. There is no expected exposure to NDTHPM from food. Based on the available information on the current use of NDTHPM in Canada, exposure to this substance from consumer products (dye in coloring paper) is expected to be negligible for the intended purpose of the product (negligible for dermal and inhalation routes of exposure during use of paper products containing this dye) and low for incidental events such as exposure via mouthing of paper by toddlers. Although the potential hazard of NDTHPM is recognized, as exposure of the general population in Canada based on the use of the substance as a paper 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

The confidence in the health effects database for NDTHPM is considered to be low due to lack of substance-specific data identified. However, relatively robust hazard

information on the potential products of azo cleavage increases the overall confidence in the human hazard profile for NDTHPM. Although evidence for reductive cleavage has been demonstrated for other azo substances, there is significant uncertainty regarding the potential for azo cleavage to occur with NDTHPM.

Confidence in the environmental exposure estimates is moderate. While no literature data were identified for concentrations in environmental media, considering no releases were reported under section 71 of under CEPA 1999, in conjunction with the conservatively modelled environmental exposure estimates, the intake values are not likely to be underestimates. Confidence in consumer product exposure estimates is moderate. While end products in current use in Canada are considered to have been addressed in the responses to a notice issued under section 71 of CEPA 1999, other assumptions such as the quantity of paper ingested by a toddler per mouthing event and bioavailability of NDTHPM from a paper were conservative.

Conclusion

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

Based on the currently available information on its potential to cause harm to human health, it is concluded that NDTHPM 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 NDTHPM does not meet any of the criteria under section 64 of CEPA 1999.

Because NDTHPM 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 health hazards 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 these substances so that new manufacture, import or use of this substance is notified and undergoes ecological and human health risk assessments.

Considerations for Follow-up

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

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Appendix 1 – Robust Study Summary

Determination of the partition coefficient for Basic Red 111 (Study Submission 2009a)

Item	Weight	Response	Mark
Reference: 13365Submission032. Determination of the partition coefficient for Basic Red 111			
Test substance: CAS RN: **118658-98-3**			
Could you repeat the experiment with available information?	5	Fair	3
Is a clear objective stated?	1	Yes	1
Is water quality characterized or identified (distilled or deionized)?	2	Yes	2
Are the results presented in detail, clearly and understandably?	3	Fair	2
Are the data from a primary source and not from a referenced article?	3	Primary	3
Was the chemical tested at concentrations below its water solubility?	5	Yes	5
Were particulates absent?	2	Not confirmed	2
Was a reference chemical of known constant tested?	3	Yes	3
Were other fate processes considered?	5	N/A	N/A
Was a control (blank) run?	3	Yes	3
Was temperature kept constant?	5	Yes (20–25°C)	5
Was the experiment done near room temperature (15–30°C)?	3	Yes	3
Is the purity of the test chemical reported (> 98%)?	3	Reported as 72%	1
Was the chemical's identity proven?	3	Yes	3
Is the source of the chemical reported?	1	No	0
Results: Octonal/water partition efficient: < 0.47 (average of two samples; estimated as the quotient of n-octanol and water solubility)			
Score:		36/42	
Degree of reliability		Satisfactory	

N/A – not applicable

Appendix 2 – Robust Study Summary

Determination of the water solubility for Basic Red 111 (Study Submission 2009b)

Item	Weight	Response	Mark
Reference: 13365Submission033. Determination of water solubility for Basic Red 111.			
Test substance: CAS RN: **118658-98-3**			
Could you repeat the experiment with available information?	5	Fair	3
Is a clear objective stated?	1	Yes	1
Is water quality characterized or identified (distilled or deionized)?	2	Yes	1
Are the results presented in detail, clearly and understandably?	3	Fair	3
Are the data from a primary source and not from a referenced article?	3	Primary	3
Was the chemical tested at concentrations below its water solubility?	5	Yes	5
Were particulates absent?	2	None observed but not confirmed	1
Was a reference chemical of known constant tested?	3	Not indicated	N/A
Were other fate processes considered?	5	N/A	N/A
Was a control (blank) run?	3	N/A	N/A
Was temperature kept constant?	5	Yes (ambient)	5
Was the experiment done near room temperature (15–30^{o} C)?	3	Yes	3
Is the purity of the test chemical reported (> 98%)?	3	Reported as 72%	1
Was the chemical's identity proven?	3	Yes	3
Is the source of the chemical reported?	1	No	0
Results: Water solubility: > 3.4 x 10^{2} g/L (average of two samples; preliminary test only)			
Score:		29/36	
Degree of reliability		Satisfactory	

N/A – not applicable

Appendix 3 – Robust Study Summary

Acute toxicity of NDTHPM to Rainbow Trout (Study Submission 2006)

No	Item	Weight	Yes/No	Specify
1	**Reference:** 13365Submission031. Acute toxicity of NDTHPM to Rainbow Trout.			
2	Substance identity: CAS RN	N/A	Y	71032-95-6
3	Substance identity: chemical name(s)	N/A	Y	
4	Chemical composition of the substance	2	Y	
5	Chemical purity	1	N	
6	Persistence/stability of test substance in aquatic solution reported?	1	Y	
7	If test material is radio-labelled, 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	
9	OECD, EU, national, or other standard method?	3	Y	- OECD Guidelines for Testing Chemicals, Section 2, Effects on Biotic Systems, Number 203, Fish Acute Toxicity Test. Updated 1983 - EEC Directive 84/449, C-1: Acute Toxicity for Fish.
10	Justification of the method/protocol if a non-standard method was used	2	N/A	
11	GLP (good laboratory practice)	3	Y	
	Test organism			
12	Organism identity: name	N/A	Y	Rainbow Trout *(Salmo gairdneri)*
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	45 mm and 1 g
16	Sex	1	N	
17	Number of organisms per replicate	1	Y	10
18	Organism loading rate	1	Y	
19	Food type and feeding periods during the acclimation period	1	Y	
	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	96 hours
23	Number of replicates (including controls)	1	Y	50
24	Concentrations	1	Y	5 testing concentrations
25	Food type/composition and feeding periods during the test	1	Y	Once daily, until 1 day before the treatment

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	N/A	
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	N/A	
28	Were concentrations in the test water measured periodically?	1	Y	
29	Were the exposure media conditions relevant to the particular chemical reported? (e.g., for the metal toxicity - pH, DOC/TOC, water hardness, temperature)	3	Y	
30	Photoperiod and light intensity	1	Y	
31	Stock and test solution preparation	1	Y	
32	Analytical monitoring intervals	1	Y	
33	Statistical methods used	1	N	
34	Was solubilizer/emulsifier used if the chemical was unstable or poorly soluble?	N/A	Y	Tween 80
	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 organism's nature/habits?	2	Y	Semi-static
38	Was pH of the test water within the range typical for the Canadian environment (6–9)?	1	Y	7.22–7.84
39	Was temperature of the test water within the range typical for the Canadian environment (5–27°C)?	1	Y	22–23°C
40	Was lipid content (or lipid-normalized BAF/BCF) reported?	2	N/A	
41	Were measured concentrations of a chemical in the test water below the chemical's water solubility?	3	Y	
42	If radio-labelled test substance was used, was BCF determination based on the parent compound (i.e., not on total radio-labelled residues)?	3	N/A	
	Results			
43	Toxicity values (specify endpoint and value)	N/A	N/A	LC_{50} (96-hr): 10–100 mg/L; based on mortality rate and concentration range tested LOEC (96-hr): > 1 mg/L NOEC (96-hr): > 1 mg/L IC_{50}: > 100 mg/L
44	Other endpoints reported – e.g., BCF/BAF, LOEC/NOEC (specify)?	N/A	Y	Estimated based on observation of clinical signs

45	Other adverse effects (e.g., carcinogenicity, mutagencity) reported?	N/A	N/A	
46	**Score:**	**36/40**		
47	**Environment Canada reliability code:**	**2**		
48	**Reliability category (high, satisfactory, low):**	**Satisfactory**		

N/A – not applicable

Appendix 4 – Robust Study Summary

Prolonged toxicity study with Rainbow Trout in a flow-through system for Basic Red 111 (Study Submission 2009g)

No	Item	Weight	Yes/No	Specify
1	**Reference:** 13365Submission034. Prolonged toxicity study with Rainbow Trout.			
2	Substance identity: CAS RN	N/A	Y	118658-98-3
3	Substance identity: chemical name(s)	N/A	Y	
4	Chemical composition of the substance	2	Y	
5	Chemical purity	1	Y	72%
6	Persistence/stability of test substance in aquatic solution reported?	1	Y	
7	If test material is radio-labelled, 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	
9	OECD, EU, national, or other standard method?	3	Y	- OECD guidelines for Testing of Chemicals, No. 203: Fish Acute Toxicity Test. April 4, 1984 - European Economic Community, EED directive 84/449, Methods for the determination of ecotoxicity. No. L251, C-1: Acute Toxicity for Fish. September 1984
10	Justification of the method/protocol if a non-standard method was used	2	N/A	
11	GLP (good laboratory practice)	3	Y	
	Test organism			
12	Organism identity: name	N/A	Y	Rainbow Trout *(Oncorhynchus mykiss)*
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	5.80 $\pm$ 0.72 cm and 2.71 $\pm$ 0.87 g
16	Sex	1	N	
17	Number of organisms per replicate	1	Y	10
18	Organism loading rate	1	Y	0.15 g of fish per litre per day
19	Food type and feeding periods during the acclimation period	1	Y	Daily with Trouvit or Artemia
	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	21 days
23	Number of replicates (including controls)	1	Y	1
24	Concentrations	1	Y	5 testing concentrations

25	Food type/composition and feeding periods during the test	1	Y	Daily with Trouvit
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	N/A	
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	N/A	
28	Were concentrations in the test water measured periodically?	1	Y	
29	Were the exposure media conditions relevant to the particular chemical reported? (e.g., for the metal toxicity - pH, DOC/TOC, water hardness, temperature)	3	Y	
30	Photoperiod and light intensity	1	Y	
31	Stock and test solution preparation	1	Y	
32	Analytical monitoring intervals	1	Y	
33	Statistical methods used	1	Y	
34	Was solubilizer/emulsifier used if the chemical was unstable or poorly soluble?	N/A	Y	
	Information relevant to the data quality			
35	Was the test organism relevant to the Canadian environment?	3	Y	
36	Were the test conditions (pH, temperature, DO, etc.) typical for the test organism?	1	Y	
37	Does system type and design (static, semi-static, flow-through; sealed or open; etc.) correspond to the substance's properties and organism's nature/habits?	2	Y	Semi-static
38	Was pH of the test water within the range typical for the Canadian environment (6–9)?	1	Y	7.8–8.3
39	Was temperature of the test water within the range typical for the Canadian environment (5–27°C)?	1	Y	14.5–15.5°C
40	Was lipid content (or lipid-normalized BAF/BCF) reported?	2	N/A	
41	Were measured concentrations of a chemical in the test water below the chemical's water solubility?	3	Y	
42	If radio-labelled test substance was used, was BCF determination based on the parent compound (i.e. not on total radio-labelled residues)?	3	N/A	
	Results			
43	Toxicity values (specify endpoint and value)	N/A	N/A	LC_{50} (21-d): 13 mg/L LC_{50} (96-hr): 16.7 mg/L
44	Other endpoints reported – e.g., BCF/BAF, LOEC/NOEC (specify)?	N/A	N/A	
45	Other adverse effects (e.g., carcinogenicity, mutagencity) reported?	N/A	N/A	
46	**Score:**	**38/40**		

47	**Environment Canada reliability code:**	**2**
48	**Reliability category (high, satisfactory, low):**	**Satisfactory**

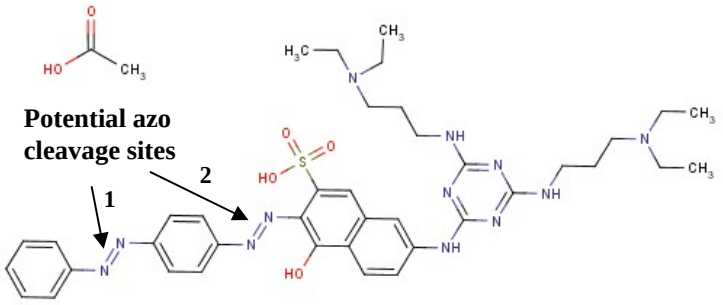
N/A – not applicable

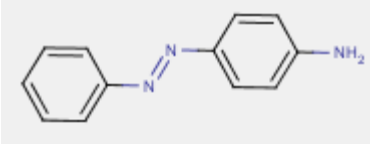
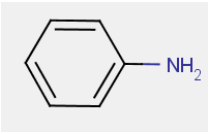
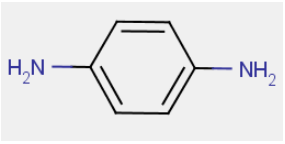
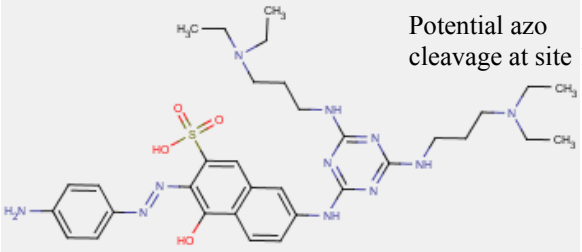
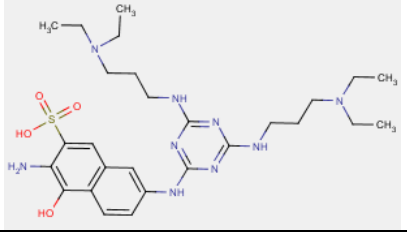
Appendix 5 – Upper-bounding Estimates of Exposure to NDTHPM from Ingestion of Non-food Use Paper by Children Aged 0.5–4 Years

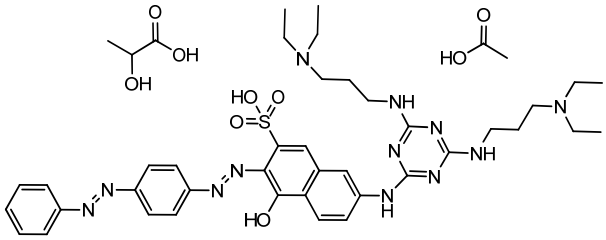
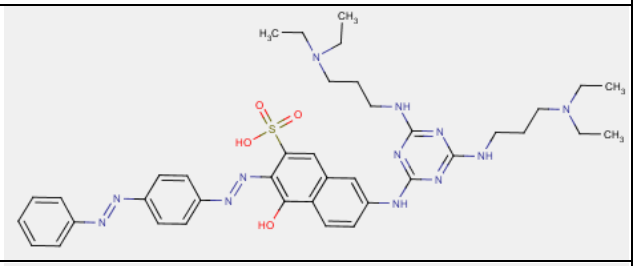
Consumer product	Assumptions	Exposure estimate (mg/kg-bw per event)
Paper (multi-use)	For standard multi-use paper, 500 sheets (dimensions 17 × 22 inches per sheet) weigh ~ 9 kg; therefore, one sheet weighs (9 kg)/500 = 0.018 kg. As a standard sheet of paper has dimensions of 8.5 × 11 inches, one standard sheet of paper weighs (0.018 kg) × [(8.5 × 11)/(17 × 22)] or ~0.0045 kg (~4.5 g). It was very conservatively assumed that ¼ of all of the NDTHPM in a sheet of paper was ingested (~ 1 g of paper). The maximum concentration of NDTHPM dye applied to paper products is 1% (2010 personal communication from Industry to Chemicals Sector Directorate, Environment Canada; unreferenced). It was assumed that the NDTHPM was completely absorbed. **Estimated oral intake:** Intake = [Concentration (w/w) of NDTHPM in paper × weight of paper eaten] / body weight For children aged 0.5–4 years[1]: Intake = [0.01 × (1 g)] / 15.5 kg = 0.6 mg/kg-bw	Oral exposure, for children aged 0.5–4 years: 0.6 mg/kg-bw per event

[1] Body weight assumed to be 15.5 kg (Health Canada 1998).

Appendix 6 – Potential Azo Cleavage Products, Related Salts and Parent Structure of NDTHPM

CAS RN 71032-95-6	Structure
NDTHPM 2-Naphthalenesulfonic acid, 7-((4,6-bis((3-(diethylamino)propyl)amino)-1,3,5-triazin-2-yl)amino)-4-hydroxy-3-((4-(phenylazo)phenyl)azo)-, monoacetate (salt)	

Basis for consideration	CAS RN Name	Structure	
Potential azo cleavage product of NDTHPM	60-09-3 4-aminoazobenzene		Potential azo cleavage at site 2
	62-53-3 aniline		Potential azo cleavage at site 1
	106-50-3 1,4-diaminobenzene or *p*-phenylenediamine (PPD)		Potential azo cleavage at sites 1 and 2
	N/A (not found in SciFinder)		Potential azo cleavage at site 1
	N/A (not found in SciFinder)		Potential azo cleavage at site 2

Basis for consideration	**CAS RN** **Name**	**Structure**
Acetate/lactate salt of NDTHPM	118658-98-3 CI Basic Red 111 (C.I. 284240)	
Parent azo structure of NDTHPM	71032-94-5 2-Naphthalenesulfonic acid, 7-((4,6-bis((3-(diethylamino)propyl)amino)-1,3,5-triazin-2-yl)amino)-4-hydroxy-3-((4-(phenylazo)phenyl)azo)-,	
Lactate salt of NDTHPM	125329-01-3 2-Naphthalenesulfonic acid, 7-((4,6-bis((3-(diethylamino)propyl)amino)-1,3,5-triazin-2-yl)amino)-4-hydroxy-3-((4-(phenylazo)phenyl)azo)-, monolactate (salt)