Government of Canada Gouvernement du Canada

Screening Assessment

Aromatic Azo and Benzidine-based Substance Grouping

Certain Azo Basic Dyes

Environment and Climate Change Canada
Health Canada

May 2016

Canada

Synopsis

Pursuant to sections 68 or 74 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999), the Ministers of the Environment and of Health have conducted a screening assessment on 33 azo basic dyes. These substances constitute a subgroup of the Aromatic Azo and Benzidine-based Substance Grouping being assessed as part of the Substance Groupings Initiative of the Government of Canada's Chemicals Management Plan (CMP) based on structural similarity and applications. Substances in this Grouping were identified as priorities for action, as they met categorization criteria under subsection 73(1) of CEPA 1999 and/or were considered as a priority based on other human health concerns.

An assessment to determine whether one basic dye (NDTHPM) met one or more criteria under section 64 of CEPA 1999 was previously conducted under the Challenge Initiative of the CMP. NDTHPM was concluded not to meet the criteria under section 64 of CEPA 1999. The Chemical Abstracts Service Registry Number (CAS RN)[1], *Domestic Substances List* (DSL) name and Colour Index (C.I.) or generic name (if applicable) of the 33 azo basic dyes are presented in the following table.

Table S1: Identity of 33 azo basic dyes in the Aromatic Azo and Benzidine-based Substance Grouping

CAS RN	***Domestic Substances List* name**	**Colour Index name or generic name**
136-40-3[a]	2,6-Pyridinediamine, 3-(phenylazo)-, monohydrochloride	Phenazopyridine hydrochloride
532-82-1 [a]	1,3-Benzenediamine, 4-(phenylazo)-, monohydrochloride	Basic Orange 2
2869-83-2	Phenazinium, 3-(diethylamino)-7-[[4-(dimethylamino)phenyl]azo]-5-phenyl-, chloride	N/A
4608-12-2	Phenazinium, 3-(dimethylamino)-7-[[4-(dimethylamino)phenyl]azo]-5-phenyl-, chloride	N/A
4618-88-6	Phenazinium, 3-amino-7-[[4-(dimethylamino)phenyl]azo]-5-phenyl-, chloride	N/A
10114-58-6	1,3-Benzenediamine, 4,4′-[1,3-phenylenebis(azo)]bis-, dihydrochloride	Basic Brown 1
10189-42-1	Pyridinium, 1-[2-[[4-[[2,6-dichloro-4-[(dimethylamino)sulfonyl]phenyl]azo]phenyl]ethylamino]ethyl]-, chloride	N/A
14408-20-9	Pyridinium, 1-[2-[[4-[(2,6-dichloro-4-nitrophenyl)azo]phenyl]ethylamino]ethyl]-, chloride	N/A

[1] The Chemical Abstracts Service Registry Number is the property of the American Chemical Society and any use or redistribution, except as required in supporting regulatory requirements and/or for reports to the government when the information and the reports are required by law or administrative policy, is not permitted without the prior written permission of the American Chemical Society.

14970-39-9	1*H*-1,2,4-Triazolium, 5-[[4-(diethylamino)phenyl]azo]-1,4-dimethyl-, trichlorozincate(1−)	N/A
23408-72-2	Benzothiazolium, 2-[[4-(dimethylamino)phenyl]azo]-3-ethyl-6-methoxy-, trichlorozincate(1−)	N/A
29508-48-3	1*H*-Pyrazolium, 1,5-dimethyl-3-[(2-methyl-1*H*-indol-3-yl)azo]-2-phenyl-, methyl sulfate	N/A
36986-04-6	Pyridinium, 1-[2-[[4-[(2-chloro-4-nitrophenyl)azo]phenyl]ethylamino]ethyl]-, chloride	N/A
52769-39-8	1*H*-1,2,4-Triazolium, dimethyl-3-[[4-[methyl(phenylmethyl)amino]phenyl]azo]-, trichlorozincate(1−)	N/A
59709-10-3	Pyridinium, 1-[2-[[4-[(2-chloro-4-nitrophenyl)azo]phenyl]ethylamino]ethyl]-, acetate	N/A
63589-49-1	1*H*-Pyrazolium, 2-cyclohexyl-3-[[4-(diethylamino)phenyl]azo]-1-methyl-, (T-4)-tetrachlorozincate(2−) (2:1)	N/A
63681-54-9	Benzenesulfonic acid, dodecyl-, compd. with 4-(phenylazo)-1,3-benzenediamine (1:1)	N/A
65150-98-3	Thiazolium, 2-[[4-(diethylamino)phenyl]azo]-3-methyl-, (T-4)-tetrachlorozincate(2−) (2:1)	N/A
68929-07-7	Benzothiazolium, 2-[[4-[ethyl(2-hydroxyethyl)amino]phenyl]azo]-5-methoxy-3-methyl-, methyl sulfate (salt)	N/A
68936-17-4	1*H*-Imidazolium, 2-[[4-(dimethylamino)phenyl]azo]-1,3-dimethyl-, (T-4)-tetrachlorozincate(2−) (2:1)	N/A
69852-41-1	Benzothiazolium, 2-[[4-[ethyl(2-hydroxyethyl)amino]phenyl]azo]-6-methoxy-3-methyl-, (T-4)-tetrachlorozincate(2−) (2:1)	N/A
71032-95-6	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
72361-40-1	Pyridinium, 1-[2-[[4-[(2-bromo-4,6-dinitrophenyl)azo]-3-methylphenyl]ethylamino]ethyl]-, chloride	N/A
72379-36-3	1*H*-1,2,4-Triazolium, 5-[[4-[ethyl(phenylmethyl)amino]phenyl]azo]-1,4-dimethyl-, (T-4)-tetrachlorozincate(2−) (2:1)	N/A
72379-37-4	1*H*-1,2,4-Triazolium, 3-[[4-[ethyl(phenylmethyl)amino]phenyl]azo]-1,2-dimethyl-, (T-4)-tetrachlorozincate(2−) (2:1)	N/A
74744-63-1	1*H*-1,2,4-Triazolium, 3,3′(or 5,5′)-[1,2-ethanediylbis[(ethylimino)-4,1-phenyleneazo]]bis[1,4-dimethyl-, (T-4)-tetrachlorozincate(2-) (1:1)	N/A
75199-20-1	1,3′-Bipyridinium, 1′,2′-dihydro-6′-hydroxy-3,4′-dimethyl-2′-oxo-5′-[[4-(phenylazo)phenyl]azo]-, chloride	N/A
75660-25-2 [a]	1,3-Benzenediamine, 4-(phenylazo)-, monoacetate	N/A
79234-33-6 [a]	1,3-Benzenediamine, 4-(phenylazo)-, acetate	N/A
83969-13-5	1,3,4-Thiadiazolium, 5-[bis(1-methylethyl)amino]-2-[[4-(dimethylamino)phenyl]azo]-3-methyl-, sulfate (2:1)	N/A
85114-37-0	1*H*-1,2,4-Triazolium, 1,4-dimethyl-3(or 5)-[[4-[methyl(phenylmethyl)amino]phenyl]azo]-, (T-4)-tetrachlorozincate(2−) (2:1)	N/A
85480-88-2	Benzothiazolium, 3-(3-amino-3-oxopropyl)-2-[(1-ethyl-2-phenyl-1*H*-indol-3-yl)azo]-, (T-4)-tetrachlorozincate(2−) (2:1)	N/A
93783-70-1	1,3,4-Thiadiazolium, 5-[bis(1-methylethyl)amino]-2-[[4-(dimethylamino)phenyl]azo]-3-methyl-, trichlorozincate(1−)	N/A
125329-01-3	Propanoic acid, 2-hydroxy-, compd. with 7-[[4,6-bis[[3-(diethylamino)propyl]amino]-1,3,5-triazin-2-yl]amino]-4-hydroxy-3-[[4-(phenylazo)phenyl]azo]-2-naphthalenesulfonic acid (1:1)	N/A

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; N/A, not applicable

[a] This substance was not identified under subsection 73(1) of CEPA 1999 but was included in this assessment as it was considered as a priority based on other human health concerns.

The 33 azo basic dyes are not expected to occur naturally in the environment. No manufacturing activity of any of the 33 azo basic dyes in Canada was reported above the 100 kg/year threshold, according to recent surveys under section 71 of CEPA 1999. Six substances have been reported as being imported into Canada above the 100 kg/year survey reporting threshold.

Environment

Azo basic dyes have moderate to high water solubility (0.1–340 g/L). Azo basic dyes are expected to settle out of the water column to bed sediments or wastewater sludge. Modelled biodegradation data for azo basic dyes predict that these substances would biodegrade slowly in water under aerobic conditions. In sediment and soil, biodegradation is also expected to be slow under aerobic conditions and fast under anaerobic conditions. Azo basic dyes may degrade and transform to certain aromatic amines if they reach anaerobic environments.

Azo basic dyes are not expected to bioaccumulate given their physical and chemical properties (i.e., low log octanol–water partition coefficients, ionized at relevant environmental pH, moderate molar weights, relatively large cross-sectional diameters and moderate to high water solubilities).

Azo basic dyes were divided into seven ecological subsets, and the critical toxicity value for the most sensitive ecological subset was derived from the most sensitive valid experimental value. Most substances had median lethal concentrations (LC_{50} values) that ranged between 0.3 and 13 mg/L for aquatic organisms. Based on the experimental and read-across data and the low critical toxicity values for each subset, it is concluded that azo basic dyes may be expected to be hazardous to aquatic organisms at moderate concentrations (i.e., LC_{50} < 10 mg/L). Based on limited empirical soil toxicity data, it is expected azo basic dyes are not expected to cause harm to soil-dwelling organisms at low concentrations.

Given that the water column is the major environmental compartment for the presence of azo basic dyes, aquatic exposure analyses were focused on scenarios representing potential major environmental releases due to industrial activities that may result in high levels of exposure of aquatic organisms. Predicted environmental concentrations were calculated for the aquatic environment for those substances used in chemical formulation, paper dyeing, textile dyeing and pharmaceutical production processes. The predicted environmental concentrations were derived in the form of probabilistic distributions due to the variability and uncertainty in several contributing variables. The probability that the predicted environmental concentrations of azo basic dyes exceeded the predicted no-effect concentration was very low in all four scenarios.

Considering all available lines of evidence presented in this Screening Assessment, there is low risk of harm to organisms and the broader integrity of the environment from the 33 azo basic dyes evaluated in this assessment. It is concluded that these azo basic dyes do not meet the criteria under paragraph 64(*a*) or 64(*b*) of CEPA 1999, as they are not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

Human Health

With respect to human health, the current Screening Assessment addresses 32 of 33 substances in the Azo Basic Dyes subgroup. The remaining substance, NDTHPM, was previously assessed and a conclusion was published under the Challenge Initiative of the CMP. As no significant new information was identified for NDTHPM, the human health risk assessment for this substance has not been updated.

Exposure of the general population of Canada to the substances in the Azo Basic Dyes subgroup from environmental media is not considered to be a significant source of exposure and therefore risk to human is considered low from environmental media.

Of the 32 Azo Basic Dyes included in the human health assessment, 12 substances have been identified as being available in certain products (paper products, textiles, drugs and cosmetics) in Canada based on available information. For two of these 12 substances (phenazopyridine hydrochloride and CAS RN 63681-54-9) although uses were reported under section 71, based on available information, exposure to the general population is not expected for these substances. Therefore, 10 of these 12 substances were considered to have potential exposure to the general population (Basic Orange 2, Basic Brown 1, CAS RNs 14408-20-9, 36986-04-6, 52769-39-8, 59709-10-3, 68929-07-7, 69852-41-1, 75660-25-2, 93783-70-1) and risk was characterized for these substances.

The margins between the estimate of dermal exposure to Basic Orange 2 in hair dye and the short-term critical health effect in rats are considered adequate to address uncertainties in the exposure and health effects databases. The margin of exposure derived for Basic Orange 2 for combined incidental oral and dermal exposure to toddlers through pen ink and the oral short-term effect level in rats is considered adequate to address uncertainties in the exposure and health effects databases.

Use of CAS RN 75660-25-2, CAS RN 52769-39-8, and Basic Brown 1 in paper products may result in potential exposure, however the risk to human health is considered to be low for this use.

Exposure to CAS RNs 14408-20-9, 36986-04-6, 59709-10-3, 68929-07-7, 69852-41-1 and 93783-70-1 may occur through dermal and oral contact with textiles as well as oral ingestion of paper. No health effects data were identified for these substances in the

Azo Basic Dyes subgroup, nor were appropriate analogues identified. There were also no indications of effects of concern for those azo cleavage products for which data was available. In the absence of suitable toxicity data for the above mentioned substances, health effect levels from phenazopyridine hydrochloride have been applied as a conservative approach, resulting in margins of exposure that are adequate to address uncertainties in the exposure and health effects databases. Therefore, for dermal exposure to textiles and incidental ingestion of textiles or paper containing these substances, the risk to human health is considered to be low.

For the remaining 20 of the 32 Azo Basic Dyes included in the human health assessment, available information did not identify sources of current exposure for the general population of Canada, therefore risk to human health is not expected for these substances.

Some of the Azo Basic Dyes in this assessment have effects of concern based on potential carcinogenicity. While available information does not indicate a risk to human health for Canadians at current levels of exposure, there may be a concern if exposures were to increase.

Based on the information presented in this Screening Assessment, it is concluded that the azo basic dyes evaluated in this assessment do not meet the criteria under paragraph 64(*c*) of CEPA 1999 as they are not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health. In addition, there are no updates to the assessment and conclusion made with respect to paragraph 64(*c*) for NDTHPM, previously considered by the Government of Canada under the Challenge Initiative of the CMP.

Overall Conclusion

It is concluded that the 33 azo basic dyes evaluated in this assessment do not meet any of the criteria set out in section 64 of CEPA 1999.

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1. Introduction

Pursuant to section 74 of the *Canadian Environmental Protection Act, 1999* (CEPA 1999) (Canada 1999), the Minister of the Environment and the Minister of Health 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 to human health as described in section 64 of the Act. In addition, under section 68 of CEPA 1999, the Minister of the Environment and the Minister of Health may conduct assessments of other substances in order to determine whether they present or may present a risk to the environment or to human health as described in section 64 of the Act.

The Substance Groupings Initiative is a key element of the Government of Canada's Chemicals Management Plan (CMP). The Aromatic Azo and Benzidine-based Substance Grouping consists of 358 substances that were identified as priorities for assessment, as they met the categorization criteria under section 73 of CEPA 1999 and/or were considered as a priority based on human health concerns (Environment Canada and Health Canada 2007;). Some substances within this Substance Grouping have been identified by other jurisdictions as a concern due to the potential cleavage of the azo bonds, which can lead to the release of aromatic amines that are known or likely to be carcinogenic.

While many of these substances have common structural features and similar functional uses as dyes or pigments in multiple sectors, diversity within the substance group has been taken into account through the establishment of subgroups. Subgrouping based on structural similarities, physical and chemical properties, and common functional uses and applications accounts for variability within this substance grouping and allows for subgroup-specific approaches in the conduct of screening assessments. This screening assessment considers substances that belong to the "Azo Basic Dyes" subgroup. Consideration of azo bond cleavage products (aromatic amines) is a key element of the human health assessment in each subgroup. Some aromatic amines, commonly referred to as EU22 aromatic amines[2], as well as associated azo dyes are restricted in other countries (EU 2006). Information on the subgrouping approach for the Aromatic Azo and Benzidine-based Substance Grouping under Canada's CMP, as well as additional background information and regulatory context, is provided in a separate document prepared by the Government of Canada (Environment Canada and Health Canada 2013).

One of the Azo Basic Dyes in this subgroup, NDTHPM (see Table 2-1 in Section 2), was previously assessed during the Challenge Initiative (Environment Canada and Health Canada 2010). NDTHPM is re-assessed as part of the present ecological risk

[2] Twenty-two aromatic amines listed in Appendix 8 of Regulation (EC) No. 1907/2006.

characterization as new ecological information is presented. Since no significant new information was identified with respect to human health risk characterization the human health effects assessment considers NDTHPM for information only.
A substance included in the Azo Basic Dyes subgroup, CAS RN 59709-10-3, was previously included as part of a screening assessment, in April 2008, of 145 persistent, bioaccumulative, and inherently toxic (PBiT) substances that were considered to be in commerce. Significant new information on ecological and human exposure has been identified for CAS RN 59709-10-3; therefore, it is included in this assessment.

Screening assessments focus on information critical to determining whether substances meet the criteria as set out in section 64 of CEPA 1999, by examining scientific information to develop conclusions by incorporating a weight of evidence approach and precaution.[3]

This screening assessment includes consideration of information on chemical properties, environmental fate, hazards, uses and exposure, including additional information submitted by stakeholders. Relevant data were identified up to August 2013. Empirical data from key studies as well as some results from models were used to reach conclusions. When available and relevant, information presented in assessments from other jurisdictions was considered.

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

This Screening Assessment was prepared by staff in the Existing Substances Program at Health Canada and Environment Canada and incorporates input from other programs within these departments. The ecological and human health portions of this assessment have undergone external written peer review and/or consultation. Comments on the technical portions relevant to the environment were received from Dr. Harold Freeman (North Carolina State University, USA) and Dr. Gisela Umbuzeiro (University of Campinas, Brazil). Comments on the technical portions relevant to human health were received from Dr. Harold Freeman (North Carolina State University, USA), Dr. David Josephy (University of Guelph, Canada), Dr. Michael Bird (University of Ottawa, Canada) and Dr. Kannan Krishnan (Université de Montréal, Canada). Additionally, the

[3] A determination of whether one or more of the criteria in 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 is not relevant to, nor does it preclude, an assessment against the hazard criteria specified in the *Hazardous Products Regulations* and the *Controlled Products Regulations* which are part of the regulatory framework for the Workplace Hazardous Materials Information Systemsfor products intended for workplace use. Similarly, a conclusion based on the criteria contained in section 64 of CEPA 1999 does not preclude actions being taken under other sections of CEPA 1999 or other Acts.

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.

The critical information and considerations upon which the Screening Assessment is based are given below.

2. Identity of Substances

This screening assessment focuses on the 33 substances belonging to the Azo Basic Dyes subgroup that is part of the Aromatic Azo and Benzidine-based Substance Grouping. This subgroup is based on structural similarities and similar applications (Environment Canada and Health Canada 2013). 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) (NDTHPM) which was previously assessed in the Challenge Initiative under CMP, is re-assessed as part of the present ecological risk characterization as new ecological information is presented. In contrast, NDTHPM is included to inform the health effects assessment and was not re-assessed.

The identities of the individual substances in this screening assessment are presented in Table 2-1. The CAS RNs, *Domestic Substances List* (DSL) names, Colour Index (C.I.) generic names, C.I. constitution numbers and chemical acronyms of these substances are presented in Table 2-1. Chemical acronyms are derived from the C.I. generic names when available; otherwise, they are based on DSL names. A list of additional chemical names (e.g., trade names) is available from the National Chemical Inventories (NCI 2012).

Table 2-1: Identity of the Azo Basic Dyes

CAS RN	DSL name	C.I. name or generic name
136-40-3	2,6-Pyridinediamine, 3-(phenylazo)-, monohydrochloride	Phenazopyridine hydrochloride
532-82-1	1,3-Benzenediamine, 4-(phenylazo)-, monohydrochloride	Basic Orange 2
2869-83-2	Phenazinium, 3-(diethylamino)-7-[[4-(dimethylamino)phenyl]azo]-5-phenyl-, chloride	N/A
4608-12-2	Phenazinium, 3-(dimethylamino)-7-[[4-(dimethylamino)phenyl]azo]-5-phenyl-, chloride	N/A
4618-88-6	Phenazinium, 3-amino-7-[[4-(dimethylamino)phenyl]azo]-5-phenyl-, chloride	N/A
10114-58-6	1,3-Benzenediamine, 4,4′-[1,3-phenylenebis(azo)]bis-, dihydrochloride	Basic Brown 1
10189-42-1	Pyridinium, 1-[2-[[4-[[2,6-dichloro-4-[(dimethylamino)sulfonyl]phenyl]azo]phenyl]ethylamino]ethyl]-, chloride	N/A
14408-20-9	Pyridinium, 1-[2-[[4-[(2,6-dichloro-4-nitrophenyl)azo]phenyl]ethylamino]ethyl]-, chloride	N/A
14970-39-9	1*H*-1,2,4-Triazolium, 5-[[4-(diethylamino)phenyl]azo]-1,4-dimethyl-, trichlorozincate(1−)	N/A
23408-72-2	Benzothiazolium, 2-[[4-(dimethylamino)phenyl]azo]-3-ethyl-6-methoxy-, trichlorozincate(1−)	N/A
29508-48-3	1*H*-Pyrazolium, 1,5-dimethyl-3-[(2-methyl-1*H*-indol-3-yl)azo]-2-phenyl-, methyl sulfate	N/A
36986-04-6	Pyridinium, 1-[2-[[4-[(2-chloro-4-nitrophenyl)azo]phenyl]ethylamino]ethyl]-, chloride	N/A
52769-39-8	1*H*-1,2,4-Triazolium, dimethyl-3-[[4-[methyl(phenylmethyl)amino]phenyl]azo]-, trichlorozincate(1−)	N/A

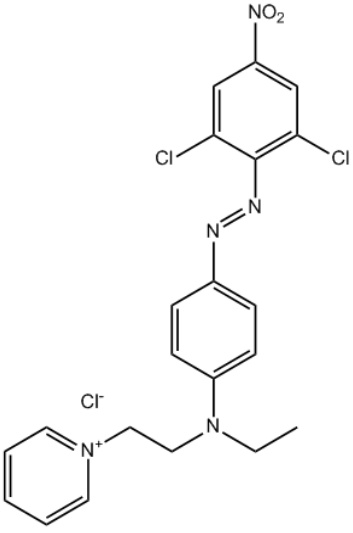
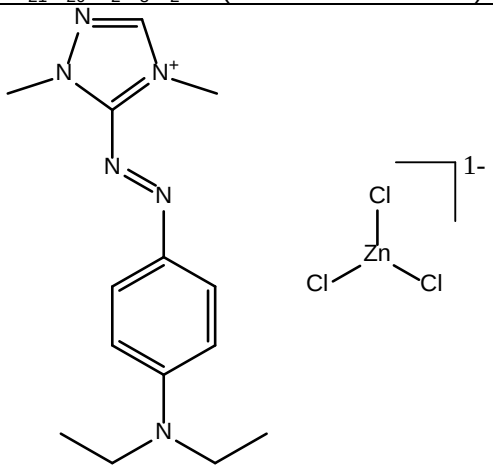
CAS RN	DSL name	C.I. name or generic name
59709-10-3	Pyridinium, 1-[2-[[4-[(2-chloro-4-nitrophenyl)azo]phenyl]ethylamino]ethyl]-, acetate	N/A
63589-49-1	1*H*-Pyrazolium, 2-cyclohexyl-3-[[4-(diethylamino)phenyl]azo]-1-methyl-, (T-4)-tetrachlorozincate(2−) (2:1)	N/A
63681-54-9	Benzenesulfonic acid, dodecyl-, compd. with 4-(phenylazo)-1,3-benzenediamine (1:1)	N/A
65150-98-3	Thiazolium, 2-[[4-(diethylamino)phenyl]azo]-3-methyl-, (T-4)-tetrachlorozincate(2−) (2:1)	N/A
68929-07-7	Benzothiazolium, 2-[[4-[ethyl(2-hydroxyethyl)amino]phenyl]azo]-5-methoxy-3-methyl-, methyl sulfate (salt)	N/A
68936-17-4	1*H*-Imidazolium, 2-[[4-(dimethylamino)phenyl]azo]-1,3-dimethyl-, (T-4)-tetrachlorozincate(2−) (2:1)	N/A
69852-41-1	Benzothiazolium, 2-[[4-[ethyl(2-hydroxyethyl)amino]phenyl]azo]-6-methoxy-3-methyl-, (T-4)-tetrachlorozincate(2−) (2:1)	N/A
71032-95-6	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
72361-40-1	Pyridinium, 1-[2-[[4-[(2-bromo-4,6-dinitrophenyl)azo]-3-methylphenyl]ethylamino]ethyl]-, chloride	N/A
72379-36-3	1*H*-1,2,4-Triazolium, 5-[[4-[ethyl(phenylmethyl)amino]phenyl]azo]-1,4-dimethyl-, (T-4)-tetrachlorozincate(2−) (2:1)	N/A
72379-37-4	1*H*-1,2,4-Triazolium, 3-[[4-[ethyl(phenylmethyl)amino]phenyl]azo]-1,2-dimethyl-, (T-4)-tetrachlorozincate(2−) (2:1)	N/A
74744-63-1	1*H*-1,2,4-Triazolium, 3,3′(or 5,5′)-[1,2-ethanediylbis[(ethylimino)-4,1-phenyleneazo]]bis[1,4-dimethyl-, (T-4)-tetrachlorozincate(2−) (1:1)	N/A
75199-20-1	1,3′-Bipyridinium, 1′,2′-dihydro-6′-hydroxy-3,4′-dimethyl-2′-oxo-5′-[[4-(phenylazo)phenyl]azo]-, chloride	N/A
75660-25-2	1,3-Benzenediamine, 4-(phenylazo)-, monoacetate	N/A
79234-33-6	1,3-Benzenediamine, 4-(phenylazo)-, acetate	N/A
83969-13-5	1,3,4-Thiadiazolium, 5-[bis(1-methylethyl)amino]-2-[[4-(dimethylamino)phenyl]azo]-3-methyl-, sulfate (2:1)	N/A
85114-37-0	1*H*-1,2,4-Triazolium, 1,4-dimethyl-3(or 5)-[[4-[methyl(phenylmethyl)amino]phenyl]azo]-, (T-4)-tetrachlorozincate(2−) (2:1)	N/A
85480-88-2	Benzothiazolium, 3-(3-amino-3-oxopropyl)-2-[(1-ethyl-2-phenyl-1*H*-indol-3-yl)azo]-, (T-4)-tetrachlorozincate(2−) (2:1)	N/A
93783-70-1	1,3,4-Thiadiazolium, 5-[bis(1-methylethyl)amino]-2-[[4-(dimethylamino)phenyl]azo]-3-methyl-, trichlorozincate(1−)	N/A
125329-01-3	Propanoic acid, 2-hydroxy-, compd. with 7-[[4,6-bis[[3-(diethylamino)propyl]amino]-1,3,5-triazin-2-yl]amino]-4-hydroxy-3-[[4-(phenylazo)phenyl]azo]-2-naphthalenesulfonic acid (1:1)	N/A

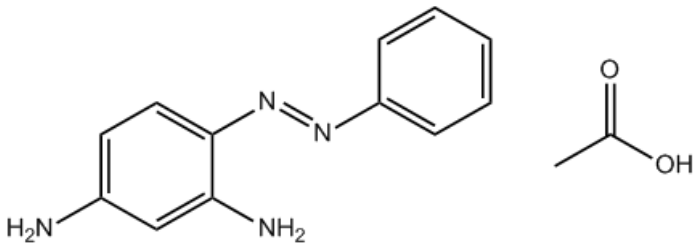
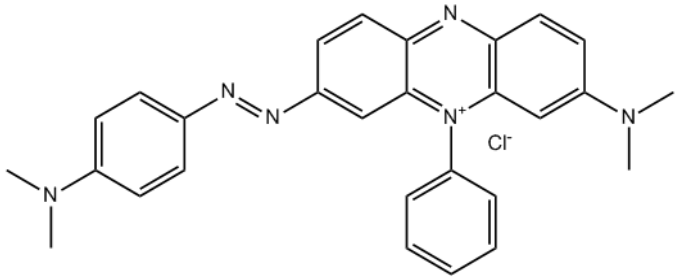
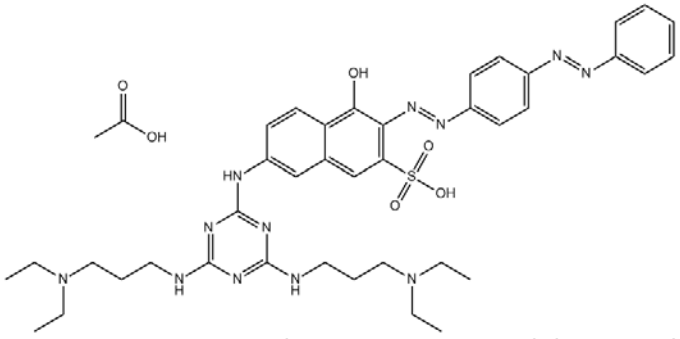
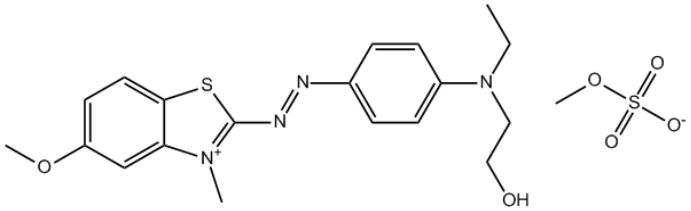
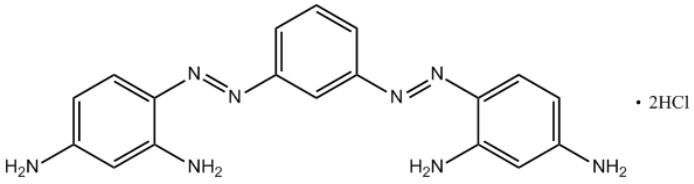
Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index; DSL, Domestic Substances List; N/A, not applicable

Example chemical structures, molecular formulas, group descriptions and molar weight ranges are presented in Table 2-2. Substances were divided into seven eco-subsets based on their chemical structures, critical functional groups (i.e., zinc salt, aryl, sulfonic acid, aniline, etc.) and molar weights. This grouping scheme permitted the use of

analogues to fill in data gaps. Subsequent analysis of the physical and chemical properties, as well as the structural similarities of the Azo Basic Dyes, allowed for a unique grouping for the assessment of environmental fate. This general approach is discussed further in Section 2.1 below. Molar weights of Azo Basic Dyes vary between 249 and 950 g/mol, with dyes of subset 3 presenting the lowest molar weight and subset 2 the highest. Subsets 2 and 6 present wider molar weight ranges, as they may contain molecules of 1:1 and 2:1 ratios of the dye molecule to its counterion. Dyes of subsets 2 and 7 may contain one or two azo bonds. Individual chemical structures, molecular formulas and molar weights are presented for all Azo Basic Dyes in Appendix A, Table A1.

Table 2-2: Example structures and descriptions for Azo Basic Dyes

Subset	Example structure for the subset	Group description with critical functional groups	Molar weight range (g/mol)
1 (*n* = 5)	 $C_{21}H_{20}Cl_2N_5O_2 \cdot Cl$ (CAS RN 14408-20-9)	Monoazo Pyridine Nitro/sulfonamide Aromatic amine Ammonium salt	446–550
2 (*n* = 13)	 $C_{14}H_{23}N_6 \cdot Cl_3Zn$ (CAS RN 14970-39-9)	Monoazo or diazo Aromatic amine Triazole/thiazole/ thiadiazole/pyrazole Zinc salt	447–1116

Subset	Example structure for the subset	Group description with critical functional groups	Molar weight range (g/mol)
3 (*n* = 4)	 $C_{12}H_{12}N_4 \cdot C_2H_4O_2$ (CAS RN 79234-33-6)	Monoazo Aniline/aminoaniline Aryl	249– 272
4 (*n* = 3)	 $C_{28}H_{27}N_6 \cdot Cl$ (CAS RN 4608-12-2)	Monoazo Phenazine Aryl	455– 511
5 (*n* = 2)	 $C_{39}H_{50}N_{12}O_4S \cdot C_2H_4O_2$ (CAS RN 71032-95-6) (NDTHPM)	Diazo Aromatic amine Melamine Naphthol Sulfonic acid	843– 873
6 (*n* = 3)	 $C_{19}H_{23}N_4O_2S \cdot CH_3O_4S$ (CAS RN 68929-07-7)	Monoazo Aryl Sulfate Benzothiazole/indole/ thiadiazole/pyrazole	444– 791
7 (*n* = 3)	 $C_{18}H_{18}N_8 \cdot 2HCl$ (CAS RN 10114-58-6) (Basic Brown 1)	Monoazo or diazo 2 or more aromatic amines	419– 539

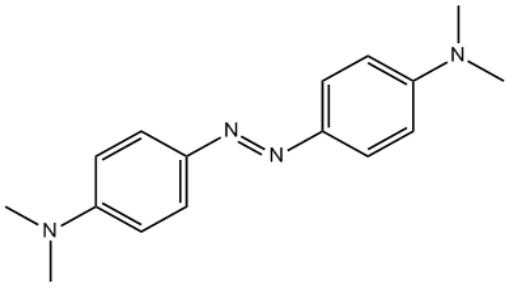
2.1 Selection of Analogues for Read-Across and Use of (Q)SAR Models

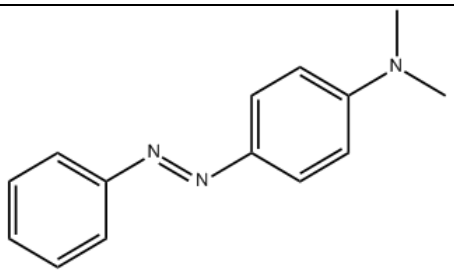
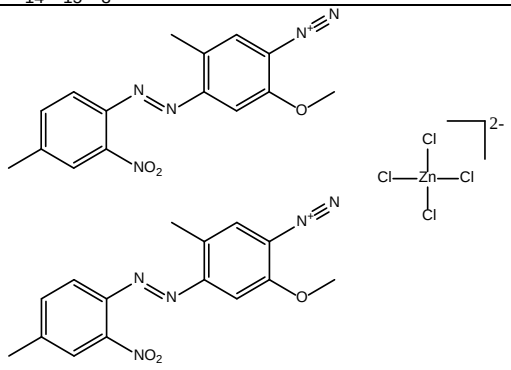
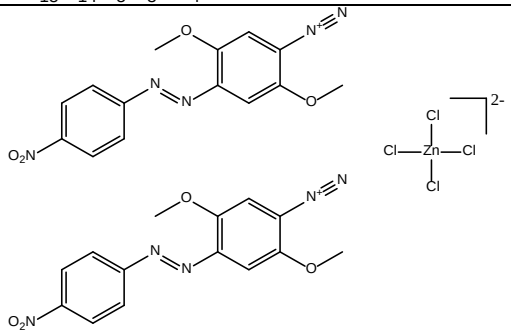
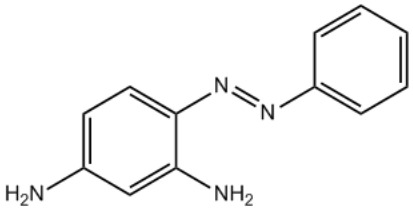
Guidance on the use of read-across approaches has been prepared by various organizations, such as the Organisation for Economic Co-operation and Development (OECD) (OECD 2014). It has been applied in various regulatory programs, including the European Union's (EU) Existing Substances Programme. The general method for analogue selection and the use of (quantitative) structure–activity relationship ((Q)SAR) models is provided in Environment Canada and Health Canada (2013). For characterization of human health effects, the basis for the use of analogues and/or (Q)SAR modelling data is documented in the Health Effects Assessment section of this report.

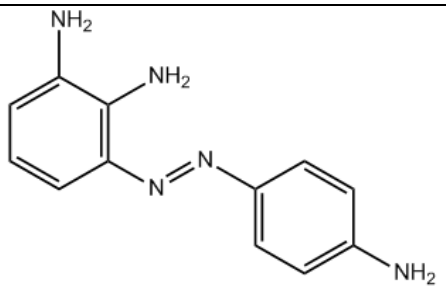
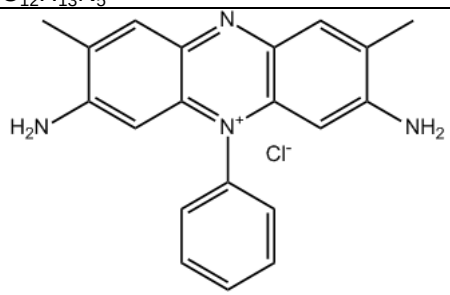
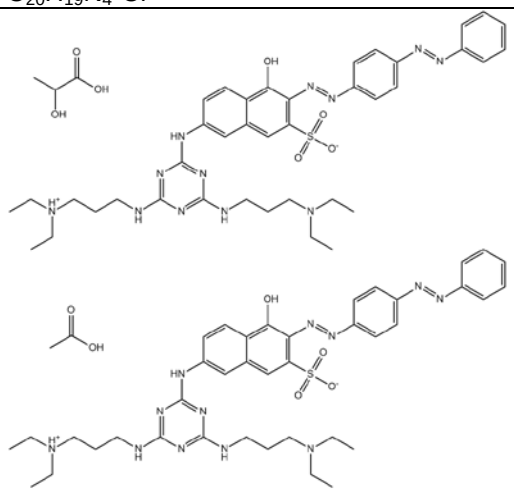
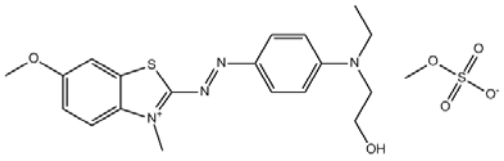
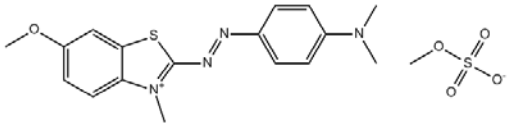
Analogues used to inform the ecological assessment were selected based on the structural similarity and availability of relevant empirical data pertaining to physical and chemical properties, persistence, bioaccumulation and ecotoxicity. Such data were used as read-across data for those Azo Basic Dyes that lacked empirical data, where appropriate, or to support the weight of evidence of existing empirical information. Although analogue data are used preferentially to fill data gaps for the substances in this assessment, the applicability of (Q)SAR models to the Azo Basic Dyes is determined on a case-by-case basis.

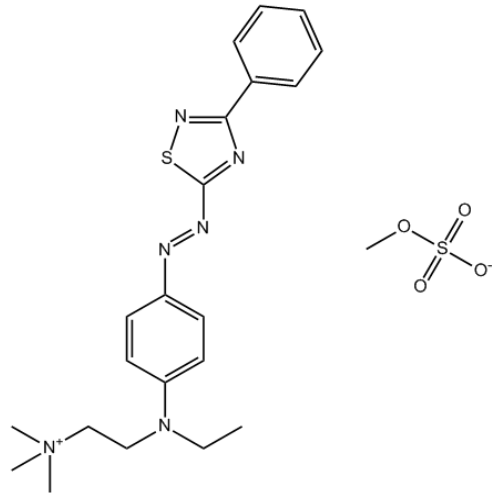
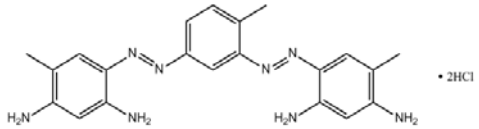
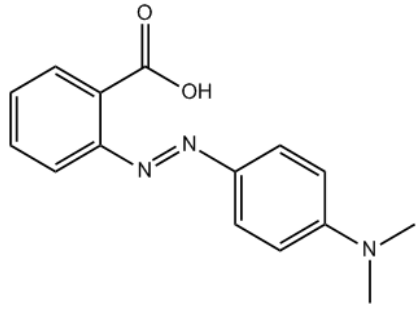
A list of the various analogues used to inform this assessment is presented in Table 2-3, along with an indication of the potential read-across data available for different parameters. All of these substances are azo compounds, most of them being azo basic dyes. More detailed information regarding the identity of these substances can be found in Appendix A (Tables A2 to A8).

Table 2-3: Identities of analogues and parameters to be used to inform the physical and chemical properties, environmental fate and potential to cause ecological harm of the Azo Basic Dyes

CAS RN / C.I. name	Chemical structure	Molar weight (g/mol)	Parameters to be used in report	Description
6257-64-3 Read-across for subset 1	 $C_{16}H_{20}N_4$	268	Melting point, boiling point, vapour pressure, log K_{ow}	Monoazo Aromatic amine

CAS RN / C.I. name	Chemical structure	Molar weight (g/mol)	Parameters to be used in report	Description
60-11-7 Read-across for subset 1	 $C_{14}H_{15}N_3$	225	Ecotoxicity	Monoazo Aromatic amine
61966-14-1 Read-across for subset 2	 $2C_{15}H_{14}N_5O_3 \cdot Cl_4Zn$	832	Melting point and solubility	Monoazo Ether Nitro Diazonium Zinc salt
64071-86-9 Read-across for subset 2	 $2C_{14}H_{12}N_5O_4 \cdot Cl_4Zn$	836	Melting point and solubility	Monoazo Ether Nitro Diazonium Zinc salt
495-54-5 Solvent Orange 3 Read-across for subset 3	 $C_{12}H_{12}N_4$	212	Ecotoxicity	Monoazo Aminoaniline Aryl

CAS RN / C.I. name	Chemical structure	Molar weight (g/mol)	Parameters to be used in report	Description
80324-43-2 Read-across for subset 3	 $C_{12}H_{13}N_5$	227	Ecotoxicity	Monoazo Aminoaniline Aniline
477-73-6 Basic Red 2 Read-across for subset 4	 $C_{20}H_{19}N_4 \cdot Cl$	351	Ecotoxicity	Phenazine Aryl
118658-98-3 Basic Red 111 Read-across for subset 5	 $2C_{39}H_{50}N_{12}O_4S \cdot 1/2C_3H_6O_3 \cdot C_2H_4O_2$	843.02–861.04	Ecotoxicity, log K_{ow}, water solubility, solubility in octanol	Diazo Aromatic amine Melamine Naphthol Sulfonic acid
12270-13-2 Basic Blue 41 Read-across for subset 6	 $C_{19}H_{23}N_4O_2S \cdot CH_3O_4S$	482	Melting point, decomposition point and biodegradation	Monoazo Aromatic amine Sulfate Benzothiazole Ether
15000-59-6 Basic Blue 54 Read-across for subset 6	 $C_{17}H_{19}N_4OS \cdot CH_3O_4S$	439	Water solubility	Monoazo Aromatic amine Sulfate Benzothiazole Ether

CAS RN / C.I. name	Chemical structure	Molar weight (g/mol)	Parameters to be used in report	Description
72906-38-8 Read-across for subset 6	 $C_{21}H_{27}N_6S{\cdot}CH_3O_4S$	507	Ecotoxicity	Monoazo Aromatic amine Aryl Thiadiazole
5421-66-9 Basic Brown 4 Read-across for subset 7	 $C_{21}H_{24}N_8{\cdot}2ClH$	461	Ecotoxicity	Diazo Aminoaniline
493-52-7 Acid Red 2 Read-across for the whole group of Azo Basic Dyes	 $C_{15}H_{15}N_3O_2$	269	Soil ecotoxicity	Monoazo Aromatic amine Aryl Carboxylic acid

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I. Colour Index; K_{ow}, octanol–water partition coefficient

3. Physical and Chemical Properties

Physical and chemical properties determine the overall characteristics of a substance and are used to determine the suitability of different substances for different applications. Such properties also play a critical role in determining the environmental fate of substances (including their potential for long-range transport), as well as their toxicity to humans and non-human organisms.

Several physical and chemical properties of Azo Basic Dyes—namely, melting point, water solubility, molecular size, log octanol–water partition coefficient (log K_{ow}) and acid dissociation constant (pK_a)—are important in terms of ecological and human health

assessment. Limited experimental data are available on the physical and chemical properties of the Azo Basic Dyes as well as their analogues. A summary of the experimental physical and chemical properties of substances that are relevant to their environmental fate and ecotoxicity is presented in Tables 3-1 to 3-7. Pivotal values, including either single mean data points (e.g., melting point and decomposition) or a range of values, have been selected to represent the properties of each subset. Detailed substance-specific information can be found in Tables A2 to A8 in Appendix A of this report.

Azo Basic Dyes carry a positive charge nitrogen atom in the molecule (Øllgaard et al. 1998). These dyes are generally characterized as having moderate to high water solubility, low vapour pressure and low log K_{ow}. Several Azo Basic Dyes are quaternary amines that are ionized at any pH, while *m*-phenylenediamine derivatives (subsets 3 and 7) are weak bases that are predominantly charged at low pH. Predicted water solubility values (using ACD/Labs Software V11.02) obtained for Solvent Orange 3 (CAS RN 495-54-5, the free base of Basic Orange 2 and related salts 63681-54-9 and 75660-25-2) (read-across for subset 3) indicate that this dye is much more water soluble at low pH (e.g., 4.0 g/L at pH 1), when the dye is in its charged form, than at higher pH (e.g., 0.030 g/L at pH 5), when the dye is in its neutral form. The modelled data is consistent with empirical observations on solubility and ionization of phenazopyridine hydrochloride which showed a water solubility at saturation of 45,000 mg/L at pH3.5 while raising the pH slightly resulted in a dramatic drop in solubility which was <40 mg/L at pH>7 (Serrajudin and Jarawaski 1985). Therefore, water solubility and ionization of these dyes is thus strongly pH dependent and helps explain the apparent wide range in water solubilities reported for these weak bases.These substances have melting points ranging from 118 to > 280°C. Cross-sectional diameters vary between 0.71 nm (minimum: D_{min}) and 1.92 nm (maximum: D_{max}), with the majority of the dyes being characterized by a diameter smaller than 1.5 nm. Analysis pertaining to cross-sectional diameter are discussed further in Section 5.5.

Table 3-1: A summary of experimental physical and chemical properties (at standard temperature of approximately 20‑25ºC) for eco-subset 1

Property	Value(s) or range (for more than 3 data points)	Pivotal value(s) for this risk assessment (basis for selection)
Solubility in water	NA	NA
Solubility in ethanol	NA	NA
Melting point	276–278°C	276–278°C (sole value)
Boiling point	> 100°C	> 100°C (sole value)
Vapour pressure	~18 mmHg[a]	18 mmHg[a] (sole value)
Log K_{ow}	2.03	2.03 (sole value)
pK_a	NA	NA
D_{min} (nm)	0.87–0.99 (*n* = 5)	Range is used (estimated values)
D_{max} (nm)	1.19–1.23 (*n* = 5)	Range is used (estimated values)

Abbreviations: D_{max}, maximum cross-sectional diameter; D_{min}, minimum cross-sectional diameter; K_{ow}, octanol–water partition coefficient; NA, not available; pK_a, acid dissociation constant

[a] 1 mmHg = 133.3 Pa.

Table 3-2: A summary of experimental physical and chemical properties (at standard temperature of approximately 20‑25ºC) for eco-subset 2

Property	Value(s) or range (for more than 3 data points)	Pivotal value(s) for this risk assessment (basis for selection)
Solubility in water	10 000 mg/L; 20 000 mg/L; 60 000 mg/L	30 000 mg/L (mean)
Solubility in *n*-octanol	3000 mg/L; 5000 mg/L	4000 mg/L (mean)
Melting point	147°C; 150°C; 149–151°C	147–151°C (range is used)
Boiling point	NA	NA
Vapour pressure	NA	NA
Log K_{ow}	NA	NA
pK_a	NA	NA
D_{min} (nm)	0.71–1.80 (n = 11)	Range is used (estimated values)
D_{max} (nm)	1.04–1.80 (n = 11)	Range is used (estimated values)

Abbreviations: D_{max}, maximum cross-sectional diameter; D_{min}, minimum cross-sectional diameter; K_{ow}, octanol–water partition coefficient; NA, not available; pK_a, acid dissociation constant

Table 3-3: A summary of experimental physical and chemical properties (at standard temperature of approximately 20–25°C) for eco-subset 3

Property	Value(s) or range (for more than 3 data points)	Pivotal value(s) for this risk assessment (basis for selection)
Solubility in water	100–1000 mg/L; 15 900 mg/L; 20 000 mg/L	100–20 000 mg/L (range is used)
Solubility in ethanol	9000 mg/L	9000 mg/L (sole value)
Melting point	118–245°C	118–245°C (range is used)
Boiling point	2262°C	2262°C (sole value)
Vapour pressure	3.51×10^{-11} mmHg[a] ; 4.57×10^{-12} mmHg[a]	1.98×10^{-11} mmHg[a] (mean)
Log K_{ow}	−0.30	−0.30 (sole value)
pK_a	NA	NA
D_{min} (nm)	NA	NA
D_{max} (nm)	NA	NA

Abbreviations: D_{max}, maximum cross-sectional diameter; D_{min}, minimum cross-sectional diameter; K_{ow}, octanol–water partition coefficient; NA, not available; pK_a, acid dissociation constant
[a] 1 mmHg = 133.3 Pa.

Table 3-4: A summary of experimental physical and chemical properties (at standard temperature of approximately 20‑25ºC) for eco-subset 4

Property	Value(s) or range (for more than 3 data points)	Pivotal value(s) for this risk assessment (basis for selection)
Solubility in water	30 000 mg/L	30 000 mg/L (sole value)

Property	Value(s) or range (for more than 3 data points)	Pivotal value(s) for this risk assessment (basis for selection)
Solubility in ethanol	5000 mg/L	5000 mg/L (sole value)
Melting point	> 200°C; 240°C	200–240°C (range is used)
Boiling point	NA	NA
Vapour pressure	NA	NA
Log K_{ow}	NA	NA
pK_a	NA	NA
D_{min} (nm)	1.09–1.15 (*n* = 3)	Range is used (estimated values)
D_{max} (nm)	1.12–1.17 (*n* = 3)	Range is used (estimated values)

Abbreviations: D_{max}, maximum cross-sectional diameter; D_{min}, minimum cross-sectional diameter; K_{ow}, octanol–water partition coefficient; NA, not available; pK_a, acid dissociation constant

Table 3-5: A summary of experimental physical and chemical properties (at standard temperature of approximately 20‑25ºC) for eco-subset 5

Property	Value(s) or range (for more than 3 data points)	Pivotal value(s) for this risk assessment (basis for selection)
Solubility in water	> 340 000 mg/L	> 340 000 mg/L (sole value)
Solubility in *n*-octanol	160 000 mg/L	160 000 mg/L (sole value)
Melting point	NA	NA
Boiling point	NA	NA
Vapour pressure	NA	NA
Log K_{ow}	< −0.33	< −0.33 (sole value)
pK_a	NA	NA
D_{min} (nm)	1.11 (*n* = 1)	1.11 (sole estimated value)
D_{max} (nm)	1.92 (*n* = 1)	1.92 (sole estimated value)

Abbreviations: D_{max}, maximum cross-sectional diameter; D_{min}, minimum cross-sectional diameter; K_{ow}, octanol–water partition coefficient; NA, not available; pK_a, acid dissociation constant

Table 3-6: A summary of experimental physical and chemical properties (at standard temperature of approximately 20‑25ºC) for eco-subset 6

Property	Value(s) or range (for more than 3 data points)	Pivotal value(s) for this risk assessment (basis for selection)

Solubility in water	140 mg/L	140 mg/L (sole value)
Solubility in ethanol	NA	NA
Melting point	> 120°C	> 120°C (sole value)
Boiling point	NA	NA
Vapour pressure	NA	NA
Log K_{ow}	NA	NA
pK_a	NA	NA
D_{min} (nm)	0.75 (*n* = 1)	0.75 (sole estimated value)
D_{max} (nm)	1.10 (*n* = 1)	1.10 (sole estimated value)

Abbreviations: D_{max}, maximum cross-sectional diameter; D_{min}, minimum cross-sectional diameter; K_{ow}, octanol–water partition coefficient; NA, not available; pK_a, acid dissociation constant

Table 3-7: A summary of experimental physical and chemical properties (at standard temperature of approximately 20‑25ºC) for eco-subset 7

Property	**Value(s) or range (for more than 3 data points)**	**Pivotal value(s) for this risk assessment (basis for selection)**
Solubility in water	< 1000 mg/L; 10 000 mg/L; 50 000 mg/L	< 1000–50 000 mg/L (range is used)
Solubility in ethanol	5000 mg/L	5000 mg/L (sole value)
Melting point	> 200°C; 200°C; > 280°C	> 200°C – > 280°C (range is used)
Boiling point	NA	NA
Vapour pressure	≤ 0.0001 kPa	≤ 0.0001 kPa (sole value)
Log K_{ow}	2	2 (sole value)
pK_a	5	5 (sole value)
D_{min} (nm)	0.75 (*n* = 1)	0.75 (sole estimated value)
D_{max} (nm)	0.82 (*n* = 1)	0.82 (sole estimated value)

Abbreviations: D_{max}, maximum cross-sectional diameter; D_{min}, minimum cross-sectional diameter; K_{ow}, octanol–water partition coefficient; NA, not available; pK_a, acid dissociation constant

4. Sources and Uses

4.1 Sources

All Azo Basic Dyes are anthropogenically produced, and consequently they do not occur naturally in the environment.

In recent years (2005 to present), all 33 substances included in this screening assessment have been included in surveys issued pursuant to section 71 of CEPA 1999 (Canada 2006a, 2009a, 2009b, 2011). These surveys aimed to collect information on manufacturing and import activities in Canada with a reporting threshold of 100 kg/year.

Based on the information received from the surveys, no manufacturing activity in Canada was reported for these Azo Basic Dyes. However, Basic Brown 1, NDTHPM, Phenazopyridine hydrochloride and CAS RNs 52769-39-8, 63681-54-9, and 75660-25-2 were reported as being imported into Canada above the reporting threshold of 100 kg/year.

Fewer than five companies reported importing a combined total of 100–1000 kg/year for CAS RNs 63681-54-9 and 52769-39-8, Basic Brown 1 and NDTHPM, and fewer than five companies reported importing a combined total of over 1000 kg/year for phenazopyridine hydrochloride and CAS RN 75660-25-2.

4.2 Uses

In general, Azo Basic Dyes are used for the dyeing of polyacrylonitrile fibres and paper (Canada 2012a). These dyes are also known to have minor uses in dyeing leather, plastics and waxes and as constituents of graphic art colours (Ullmann's Encyclopedia 2010).

Table 4-1 presents a summary of the predominant uses of the Azo Basic Dyes in Canada based on Consumer and Commercial Codes submitted in response to recent section 71 surveys (Environment Canada 2009, 2012); some reported uses are not included in Table 4-1 due to confidentiality.

Table 4-1: Summary of the major uses of certain Azo Basic Dyes in Canada based on Consumer and Commercial Codes (indicated in parentheses) submitted in response to section 71 surveys (Environment Canada 2009, 2012)[a]

CAS RN / C.I. name	Drugs (C563)	Arts, Crafts, and Hobby Materials (C305)	Paper Products (C302)	Ink, Toner, and Colourants (C306)
Basic Brown 1	No	No	Yes	No
Phenazopyridine hydrochloride	Yes	No	No	No

CAS RN / C.I. name	Drugs (C563)	Arts, Crafts, and Hobby Materials (C305)	Paper Products (C302)	Ink, Toner, and Colourants (C306)
52769-39-8	No	No	No	Yes
75660-25-2	No	Yes	No	No

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index
[a] A Consumer and Commercial Code was also submitted for CAS RN 63681-54-9 but is not indicated in this table due to confidentiality.

In Canada, food colouring agents are regulated as food additives under the *Food and Drug Regulations*. Colours that are permitted for use in food are listed in the *List of Permitted Colouring Agents* incorporated by reference in the *Marketing Authorization for Food Additives that May be Used As Colouring Agents*, issued under the authority of the *Food and Drugs Act* (Canada 2012b). None of the substances in this screening assessment are listed in the *List of Permitted Colouring Agents* as a permitted food colouring agent. One substance, Basic Brown 1, was identified for use in food packaging applications in Canada for direct contact with dry foods at room temperature. Minimal contact is expected, and exposure is expected to be negligible (personal communications, emails from Food Directorate [Health Canada] to Risk Management Bureau [Health Canada], dated 2011; unreferenced).

Of the 33 substances in this screening assessment, one substance was identified as being present in human pharmaceuticals (DPD 2013; personal communications, emails from Therapeutic Products Directorate [Health Canada] to Risk Management Bureau [Health Canada], dated 2011 and 2013; unreferenced). The active ingredient, phenazopyridine hydrochloride, was identified in prescription pharmaceuticals for humans, where it is used as a urinary tract analgesic (DPD 2013). Phenazopyridine hydrochloride is available in 100 and 200 mg tablets, with an adult dose of 200 mg three times daily, where treatment usually should not exceed 2 days (Erfa Canada Inc. 2010). The use of phenazopyridine hydrochloride as a pharmaceutical product has less than 0.10 kg per year in use in Canada, reported in 2011 and 2012 (MIDAS 2013). None of the 33 substances in this screening assessment was identified as being present in biologics (personal communication, email from Biologics and Genetic Therapies Directorate [Health Canada] to Risk Management Bureau [Health Canada], dated 2011; unreferenced) or veterinary drugs in Canada (personal communication, email from Veterinary Drugs Directorate [Health Canada] to Risk Management Bureau [Health Canada], dated 2011; unreferenced). None of the 33 substances in this screening assessment are listed in the Natural Health Products Ingredients Database as an ingredient in natural health products (NHPID 2011) or in the Licensed Natural Health Products Database as being present in currently licensed natural health products (LNHPD 2008).

Based on notifications submitted under the *Cosmetic Regulations* to Health Canada, Basic Orange 2, is used in certain cosmetic products in Canada such as hair dyes (personal communications, emails from Consumer Product Safety Directorate [Health Canada] to Existing Substances Risk Assessment Bureau [Health Canada], dated 2011

and 2013; unreferenced). The 33 substances substances in this screening assessment are not included on Health Canada's Cosmetic Ingredient Hotlist (Health Canada 2011b), an administrative tool that Health Canada uses to communicate to manufacturers and others that certain substances, when present in a cosmetic, may contravene (a) the general prohibition found in section 16 of the Food and Drugs Act or (b) a provision of the *Cosmetic Regulation*. However, a related salt of Basic Orange 2, chysoidine citrate hydrochloride (CAS RN 5909-04-6) is included in Health Canada's Cosmetic Ingredient Hotlist (Heath Canada, 2011b).

Basic Orange 2 was also identified as an ingredient in ink of ballpoint pens sold in Canada. Ten substances in this screening assessment (Basic Brown 1, Basic Orange 2, and CAS RNs 14408-20-9, 36986-04-6, 52769-39-8, 59709-10-3, 68929-07-7, 69852-41-1, 75660-25-2 and 93783-70-1) were identified as being used in Canada, based on information submitted by the Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers (ETAD) (personal communication, email from ETAD to Environment Canada, dated 2010; unreferenced). Specific uses for these substances were not identified.

5. Environmental Fate

The environmental fate of chemicals describes the processes by which the chemicals become distributed and are transformed in the environment. In this section, some general characteristics of the substances considered in this screening assessment will be discussed with respect to their environmental fate in different compartments in an effort to understand how organisms come into contact with the substances in a particular medium, the persistence of the substances in environmental compartments, and their degradation, distribution among media, migration in groundwater, removal from effluents by standard wastewater treatment methods and bioaccumulation in organisms.

As explained in Environment Canada and Health Canada (2013), mass balance fate models, such as the New Equilibrium Criterion model (New EQC 2011), are not applicable for positively charged basic dyes, as the dyes do not fall under the model domain. Therefore, the environmental fate and compartmentalization of these substances will be discussed qualitatively using information on their physical and chemical properties.

5.1 Water and Sediment

When released to the aquatic environment, Azo Basic Dyes are expected to be found mostly in the water column due to their solubility. If released to natural waters or wastewater in an untransformed state, positively charged Azo Basic Dyes are expected to have an affinity for ionized substrates, such as dissolved organic material, due to the presence of humic and fulvic acids or clays, which have a net negative surface charge (ETAD 1995; Environment Canada and Health Canada 2013). Azo Basic Dyes are expected to eventually settle out of the water column to bed sediments or, when released in wastewater, to treatment sludges. Some ionic dyes can also bind to organic material via hydrogen bonds and van der Waals forces (Oster 1955).

Other factors, such as increasing molecular size, hardness of the water and salinity, as well as decreasing pH, are thought to favour some sorption of azo dyes to suspended solids (HSDB 1983– ; Øllgaard et al. 1998). It has been stated generally that, due to the recalcitrant nature of azo dyes in aerobic environments, they eventually end up in anaerobic sediments, shallow aquifers and groundwater (Razo-Flores et al. 1997). Some Azo Basic Dyes may not be irreversibly bound to sediment or wastewater sludge and can thus be reintroduced to pore water or the water column, whereas others will bind irreversibly and remain sequestered.

5.2 Soil

Azo Basic Dyes may be released indirectly to soil via the application of wastewater biosolids to agricultural land or deposition in landfills. It is expected that ionic dyes will have high to moderate mobility in soil due to low soil–water adsorption coefficient (K_d) values (Øllgaard et al. 1998); this is tempered with the finding that they may also

undergo ion exchange processes with clay in soil, which would retard leaching (HSDB 1983–).

5.3 Air

Azo Basic Dyes are not expected to be released to air and are not expected to partition to this compartment due to their very low vapour pressures and moderate to high water solubilities (HSDB 1983– ; Øllgaard et al. 1998). Water-soluble dyes such as Azo Basic Dyes are intended for use in water-based treatments, which also limits their release, as they are hydrophilic. While premixed dyes in their solid states may have some limited capacity for dispersal into the air as large particles, air is not considered to be a carrying medium for dyes, as these substances exhibit low or negligible volatility (ETAD 1995; Øllgaard et al. 1998).

Given low levels of volatility and physicochemical preference for partitioning to other media, it is also not expected that Azo Basic Dyes will be subject to long-range atmospheric transport.

5.4 Environmental Persistence

To characterize the environmental persistence of Azo Basic Dyes, empirical and modelled data for these substances under both aerobic and anaerobic conditions were considered.

5.4.1 Empirical data for persistence

Empirical biodegradation data related to the persistence of Azo Basic Dyes are limited. Biodegradation data were available for only one substance in this assessment. A study evaluated the inherent biodegradation of CAS RN 71032-95-6 (NDTHPM) by wastewater bacteria (without pre-adaptation) using a modified Ministry of International Trade & Industry of Japan (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 (20%) was also observed in the flask without inoculum after 28 days. This study is based on inherent biodegradation tests, which generally provide favourable conditions for biodegradation compared with ready biodegradation tests.

Empirical biodegradation data were identified for two structurally related analogues, which can be used as read-across information. A study reported the inherent biodegradation of Basic Red 111 (72% purity) using a modified Zahn-Wellens test and microorganisms from a publically-owned wastewater treatment plant (Study Submission 2009a). Results showed that the test substance was not completely dissolved at the beginning of the test; after 7 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 7 of exposure. Therefore, it was not clear whether the test substance was biodegraded or adsorbed to the glass covers.

As part of a study conducted by Huren et al. (1994), an activated sludge system was used to test the aerobic biodegradability of Basic Blue 41. Results of the aerobic biodegradability test showed that the dye removal rate was higher than 70% and that the substance was readily biodegradable. Basic dyes, which are cationic, are adsorbed readily to negatively charged surfaces (i.e., sludge), thus explaining why these substances have an elevated removal rate.

5.4.2 Modelling of persistence

In addition to the experimental data, a (Q)SAR-based weight of evidence approach (Environment Canada 2007b) was applied using biodegradation models. These models are considered acceptable for use, as they are based on chemical structure, and the azo structure is represented in the training sets of all the BIOWIN models used, thereby increasing the reliability of the predictions. Given the ecological relevance of the water compartment, the fact that most of the available models apply to water and the fact that Azo Basic Dyes are expected to be released to this compartment, aerobic biodegradation in water was primarily examined.

Table A9 in Appendix A summarizes the results of available (Q)SAR models for degradation in various environmental compartments. Degradation models used in this analysis were HYDROWIN (2010), BIOWIN Submodels 3–6 (BIOWIN 2010), DS TOPKAT (©2005–2009), AOPWIN (2010) and CATALOGIC (2012).

Model outputs for Azo Basic Dyes consistently predicted that these substances would biodegrade slowly in water under aerobic conditions (Appendix A, Table A9). These results are consistent with information included in Environment Canada and Health Canada (2013), which outlines the general persistence of azo dyes in aerobic environments.

Modelled half-lives of 0.025–1.081 days for the Azo Basic Dyes indicate that these substances rapidly photooxidize via reaction with hydroxyl radicals (AOPWIN 2010). The only compound for which persistence was significant in air is phenazopyridine hydrochloride, with a modelled half-life of 4.273 days.

5.4.3 Aerobic biodegradation

While the experimental persistence data for Azo Basic Dyes were inconclusive, the modelled persistence data for Azo Basic Dyes show little to no biodegradation in the time scale of the studies. This is consistent with the understanding that dyes must be stable in order to be effective in their applications and that most are generally considered non-degradable under environmentally relevant aerobic conditions (Pagga and Brown 1986; ETAD 1995; Øllgaard et al. 1998).

5.4.4 Anaerobic biodegradation

Under anaerobic and anoxic conditions, many azo dyes are vulnerable to bacteria-mediated cleavage of their azo bond (Brown and Laboureur 1983; Baughman and Weber 1994; Weber and Adams 1995). However, complete degradation of the aromatic azo- and benzidine-based substances may not always occur, since metabolites resulting from azo bond cleavage may persist under anaerobic conditions (Pinheiro et al. 2004).

5.4.5 Hydrolysis

The majority of the Azo Basic Dyes do not contain functional groups expected to undergo hydrolysis. This is consistent with published studies that note hydrolysis as being an insignificant factor in the cleavage of azo compounds (Baughman and Perenich 1988). However, CAS RN 75199-20-1 contains an amide functional group that was flagged by EPI Suite (2012) as having the potential to undergo some degree of hydrolysis.

5.4.6 Summary of persistence

Due to the persistence of Azo Basic Dyes in aerobic environments in combination with their moderate to high water solubility, it is expected that these substances will have relatively long residence times in water. As these substances are predicted to stay in the water for long periods of time, they may disperse widely from point sources of release. Eventually, due to electrostatic interactions with negatively charged particulate matter, they will be deposited to sediment, where they will persist under aerobic conditions and remain a source of exposure to organisms until buried due to sedimentation. Deeper layers of sediment are likely under anaerobic conditions, which will transform (reduce) the dyes via azo hydrolysis. Exposure of the benthos under anaerobic conditions is not expected to be significant. Short residence times in air are expected to result in low potential for long-range atmospheric transport.

5.5 Potential for Bioaccumulation

In this assessment, a variety of lines of evidence have been used to determine the bioaccumulation potential of Azo Basic Dyes. Experimental data for traditional bioaccumulation metrics such as bioconcentration factor (BCF) are minimal and mostly restricted to the water compartment for these substances. In addition, the use of (Q)SAR bioaccumulation modelling was not pursued for Azo Basic Dyes, since these substances were outside the model domains of applicability.

5.5.1 Octanol–water partition coefficient

As indicated in Tables 3-1 to 3-7, Azo Basic Dyes have moderate to high water solubility (100–60 000 mg/L), with only the read-across for eco-subset 5 showing a very high water solubility of > 340 000 mg/L. Limited experimental log K_{ow} values (< −0.33 to 2.03)

suggest a low bioaccumulation potential according to equilibrium partitioning theory. This is consistent with the general view from other sources that ionic dyes have a very low bioaccumulation potential (ETAD 1995).

5.5.2 Aquatic bioconcentration factor (BCF)

No experimental data were available for the Azo Basic Dyes included in this assessment. However, data from Anliker et al. (1981) show that the very water soluble ionic dyes tend to have low log BCF values of approximately −1 to 1. It was suggested that this low bioaccumulation potential is a result of these dyes adhering to the outside of the fish or to the intestine; being water soluble, these dyes do not readily cross the water–lipid bilayers of cells.

5.5.3 Other factors for assessing bioaccumulation potential

As outlined in the Potential for Bioaccumulation section of Environment Canada and Health Canada (2013), due to the lack of empirical bioaccumulation data available for Azo Basic Dyes, available data on water solubility, molar weight and cross-sectional diameter are considered in order to provide evidence of bioaccumulation potential. Given their relatively high water solubility, ionic nature and high degree of dissociation under typical environmental conditions, the lipid partitioning tendency of these substances is expected to be limited. Also, bioaccumulation data resulting from exposures of organisms to these substances in soil and sediment are minimal and limited, in large part due to the moderate to high water solubility of these substances (Environment Canada and Health Canada 2013).

In general, Azo Basic Dyes are relatively hydrophilic, relatively large molecules with relatively high molar weight (249–950 g/mol). The minimum and maximum cross-sectional diameters for Azo Basic Dyes range from 0.71 nm (D_{min}) to 1.92 nm (D_{max}) (Tables 3-1 to 3-7). These characteristics suggest that molecular dimensions may also restrict the rate of uptake of these substances when crossing cell membranes in fish from water, thereby reducing the bioaccumulation potential for these substances.

5.5.4 Summary of bioaccumulation potential

Azo Basic Dyes are expected to have a low bioaccumulation potential due to low observed bioconcentration in empirical tests with other ionic dyes. This is supported by, and consistent with, their physical and chemical properties (i.e., low log K_{ow} values, ionized at relevant environmental pH, moderate molar weights, relatively large cross-sectional diameters, moderate to high water solubilities) and likely high degree of biotransformation by organisms. It is also possible that these dyes are readily metabolized in organisms via Phase I and II mechanisms, given that these dyes are known to undergo azo hydrolysis *in vivo*.

6. Potential to Cause Ecological Harm

6.1 Ecological Effects Assessment

In general, cationic dyes are known to have high levels of ecotoxicity that exceed those of acid and direct dyes (Øllgaard et al. 1998). To assess the ecological effects potential of Azo Basic Dyes, only empirical data (from specific substances within the subsets and analogues) were considered, given the high level of uncertainty associated with modelling the ecotoxicity of these substances.

6.1.1 Empirical studies for the aquatic compartment

Limited empirical studies were available for Azo Basic Dyes (Table 6-1). Most substances had very few or no empirical data, with median lethal concentrations (LC_{50}s) typically between 0.3 and 13 mg/L. Most of the studies were of short-term duration, and the majority of toxicity tests were conducted on fish species.

An LC_{50} of 0.6 mg/L (48 hours) for medaka fish (*Oryzias latipes*) exposed to CAS RN 60-11-7 (Tonogai et al. 1982) served as read-across for eco-subset 1.

A study conducted by Güngördü et al. (2013) assessed the ecotoxicity of the dye CAS RN 52769-39-8 (eco-subset 2). Groups of 15 *Xenopus laevis* tadpoles of the 46th stage were exposed to various concentrations of the dye for 7 days (168 hours). Results showed LC_{50}s of 1.57 mg/L after a 72-hour exposure and 0.35 mg/L after 168 hours. The study also reported an increase in carboxylesterase and glutathione *S*-transferase enzymatic activities.

Two empirical studies were available for three compounds from eco-subset 3. Tonogai et al. (1982) reported an LC_{50} of 0.3 mg/L (48 hours) for Solvent Orange 3 and LC_{50}s of 0.8 mg/L (24 hours) and 0.5 mg/L (48 hours) for CAS RN 80324-43-2 (read-across data). The second study, conducted by Brown et al. (1981), measured the respiratory rate of activated sludge to determine the potential inhibitory effect of various dyes, including Basic Orange 2, on aerobic wastewater bacteria. The median inhibitory concentration (IC_{50}) was 10–100 mg/L.

An LC_{50} of 7 mg/L (48 hours) for medaka fish (*Oryzias latipes*) exposed to Basic Red 2 (Tonogai et al. 1982) served as read-across for eco-subset 4. It should be noted that Basic Red 2 does not contain an azo bond; however, based on overall structural similarity with the other compounds in eco-subset 4, it is expected to express comparable properties.

Ecotoxicity studies were available for the two substances included in eco-subset 5. An empirical study exposed rainbow trout to NDTHPM (mixed with Tween 80) over a 96-hour period in a static system (Study Submission 2006). No mortality was observed in the control with Tween 80, at 0.1 mg/L or at 1 mg/L, whereas 10% mortality was

observed at 10 mg/L. All test organisms died at the highest concentration tested (100 mg/L). The study also showed a wastewater bacteria respiratory inhibitory concentration (IC_{50}) of > 100 mg/L. Empirical studies for Basic Red 111, used as a read-across in the eco-subset, also reported ecotoxicity effects on *Danio rerio*, *Daphnia magna* and *Oncorhynchus mykiss* (Study Submission 2009b, 2009c, 2009d).

Empirical ecotoxicity data were available for two of the substances included in eco-subset 6. Green alga *Selenastrum capricornutum* exposed to CAS RN 68929-07-7 had a 96-hour median effective concentration (EC_{50}) of 0.025 mg/L (Greene and Baughman 1996). Knacker et al. (1995) conducted acute ecotoxicity tests on zebrafish (*Brachydanio rerio*), acute immobilization tests on *Daphnia magna* and algal growth inhibition tests on *Scenedesmus subspicatus* using CAS RN 72906-38-8 (read-across). Data obtained from these tests were used to estimate median lethal, median effective and half-maximal inhibitory concentrations using an arcsin transformation. Two additional studies tested the ecotoxicity of CAS RN 72906-38-8 on the protozoan *Tetrahymena thermophila.* Effective concentrations ranged from 0.5 to 30 mg/L, and a no-observed-effect concentration (NOEC) of 4 mg/L for a 48-hour exposure was reported (Pauli et al. 1993, 1994). It should be noted that CAS RN 72906-38-8 contains a quaternary ammonium functional group that may render it more toxic than the dyes in subset 6, which do not contain this group.

Empirical studies were available for Basic Brown 4, a read-across substance for eco-subset 7. Ericson (1977) conducted algal assays based upon adenosine triphosphate measurement and reported cell reproduction inhibition of *Selenastrum capricornutum* at a concentration of 10 mg/L and a significant luciferase inhibition with increasing concentration of the dye. The ecotoxicity of Basic Brown 4 was also tested by Little and Lamb (1972, 1974), who reported an LC_{50} of 5.6 mg/L for fathead minnow (*Pimephales promelas*) exposed for 96 hours. Brown et al. (1981) also reported that the half-maximal inhibitory concentration (IC_{50}) of Basic Brown 4 on aerobic wastewater bacteria was > 100 mg/L. A Material Safety Data Sheet for the basic dye CAS RN 63681-54-9 reported an LC_{50} of 1–10 mg/L for the zebrafish (*Brachydanio rerio*) after an exposure of 96 hours, a EC_{50} of 1–10 mg/L for *Daphnia magna* after an exposure of 48 hours and an EC_{10} of > 10 mg/L for the inhibition of bacteria in effluent after 16 hours (BASF Canada 2004), but the empirical study that supported these data could not be obtained.

Even though there were a few algal tests reported above, the results of ecotoxicological studies for coloured substances such as dyes are not believed to be representative of ecosystem effects. The algal growth inhibition test is one of the most common tests for determining aquatic toxicity, by measuring changes in the growth rate in response to exposure to test chemicals in water. When testing coloured substances, it has been noted that these substances are capable of attenuating light penetration into the test medium, by light absorption and reflection. Using solvents or emulsifiers to create a homogeneous dispersion in water, attenuation of light is likely to be proportional to the amount of substance added. The inhibition of algal growth due to light attenuation can result in reduced algal population growth in relation to the amount of substance added

to the test medium. However, such inhibition is not considered a true ecotoxicological effect of the test chemicals (Rufli et al. 1998; Cleuvers and Weyers 2003).

There have been recommendations to deal with light attenuation in algal tests with coloured substances. It was suggested to put the algae back into a test substance–free medium after the end of the exposure period, in order to discriminate between an algistatic and an algicidal effect (Whitehouse and Mallett 1993); a reduced light path through the test solution was also proposed, so that the algal growth rate is not affected (Comber et al. 1995). However, in studies identified to assess the ecological effects of Azo Basic Dyes and analogues in algae, there is no report of any light attenuation and hence no indication as to whether light attenuation has been impacted. Therefore, it is suspected that the reported ecotoxicity in the algae studies may not represent the "true" effects of the test dyes on the organisms.

Table 6-1: Empirical data for aquatic ecotoxicity of representative substances for the Azo Basic Dyes

Subset	Test organism	Type of test (duration)	Endpoint	Value (mg/L) (CAS RN / generic name)	Reference
1	Fish (*Oryzias latipes*)	Acute (48 h)	LC_{50}	0.6 (60-11-7)	Tonogai et al. 1982
2	Tadpoles (*Xenopus laevis*)	Chronic (168 h)	LC_{50}	0.35 (52769-39-8)	Güngördü et al. 2013
3	Fish (*Oryzias latipes*)	Acute (48 h)	LC_{50}	0.3 (495-54-5)	Tonogai et al. 1982
3	Fish (*Oryzias latipes*)	Acute (48 h)	LC_{50}	0.5 (80324-43-2)	Tonogai et al. 1982
4	Fish (*Oryzias latipes*)	Acute (48 h)	LC_{50}	7 (477-73-6)	Tonogai et al. 1982
5	Fish (*Oncorhynchus mykiss*)	Acute (96 h)	LC_{50}	> 10 and < 100 (71032-95-6; NDTHPM)	Study Submission 2006
5	Wastewater bacteria	Respiratory inhibition test	IC_{50}	> 100 (71032-95-6; NDTHPM)	Study Submission 2006
q	Fish (*Danio rerio*)	Acute (96 h)	LC_{50}	16.7 (118658-98-3)	Study Submission 2009b
5	Fish (*Danio rerio*)	Acute (96 h)	NOEC	10 (118658-98-3)	Study Submission 2009b
5	*Daphnia magna*	Acute (48 h)	EC_{50}	109 (118658-98-3)	Study Submission 2009c
5	*Daphnia magna*	Acute (48 h)	NOEC	32 (118658-98-3)	Study Submission 2009c
5	Fish (*Oncorhynchus mykiss*)	Chronic (21 days)	LC_{50}	13 (118658-98-3)	Study Submission 2009d

5	Fish (*Oncorhynchus mykiss*)	Chronic (21 days)	LOEC	6 (118658-98-3)	Study Submission 2009d
5	Fish (*Oncorhynchus mykiss*)	Chronic (21 days)	NOEC	2.7 (118658-98-3)	Study Submission 2009d
6	Green alga (*Selenastrum capricornutum*)	Chronic (96 h)	EC_{50}	0.025 (68929-07-7)	Greene and Baughman 1996
6	Fish (*Brachydanio rerio*)	Acute (96 h)	LC_{50}	12.6 (72906-38-8)	Knacker et al. 1995
6	*Daphnia magna*	Acute (48 h)	EC_{50}	13.4 (72906-38-8)	Knacker et al. 1995
6	Algae (*Scenedesmus subspicatus*)	Algal growth inhibition (96 h)	IC_{50}	1.48 (72906-38-8)	Knacker et al. 1995
7	Algae (*Selenastrum capricornutum*)	Chronic (7 days)	Cell reproduction	10 (5421-66-9)	Ericson 1977
7	Fish (*Pimephales promelas*)	Acute (96 h)	LC_{50}	5.6 (5421-66-9)	Little and Lamb 1972, 1974
7	Wastewater bacteria	Respiratory inhibition test	IC_{50}	> 100 (5421-66-9)	Brown et al. 1981
7	Fish (*Brachydanio rerio*)	Acute (96 h)	LC_{50}	1–10 (63681-54-9)	BASF Canada 2004
7	*Daphnia magna*	Acute (48 h)	EC_{50}	1–10 (63681-54-9)	BASF Canada 2004

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; EC_{50}, the concentration of a substance that is estimated to cause some effect on 50% of the test organisms; IC_{50}, half-maximal inhibitory concentration; LC_{50}, the concentration of a substance that is estimated to be lethal to 50% of the test organisms; LOEC, lowest-observed-effect concentration; NOEC, no-observed-effect concentration

6.1.2 Empirical studies for other environmental compartments

Soil ecotoxicological data were reported for Acid Red 2 (Sharma et al. 2009), a dye sharing structural similarities with Azo Basic Dyes. An 8-day EC_{50} study resulted in a moderate toxicity value of 56 mg/kg of soil for lettuce (*Lactuca sativa*) (Table 6-2). No empirical data were available on the toxicity of Azo Basic Dyes in sediment. Furthermore, no suitable analogues were identified that had sediment toxicity data.

Table 6-2: Empirical soil ecotoxicity data from read-across CAS RN 493-52-7

Test organism	Type of test	Endpoint	Value (mg/kg of soil)	Reference
Lettuce (*Lactuca sativa*)	Chronic (8 days)	EC_{50} (reproduction) (soil)	56	Sharma et al. 2009

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; EC_{50}, the concentration of a substance that is estimated to cause some effect on 50% of the test organisms

6.2 Derivation of the Predicted No-Effect Concentration (PNEC)

6.2.1 Derivation of the aquatic predicted no-effect concentration (PNEC)

Due to similar toxicities among the seven eco-subsets, a single critical toxicity value (CTV) in the aquatic medium was chosen to represent the subgroup.

The aquatic CTV selected for the Azo Basic Dyes was obtained from the read-across substance, Solvent Orange 3, because it was one of the most sensitive valid experimental values. A chronic toxicity test of 168 hours had an LC_{50} of 0.35 mg/L for *Xenopus laevis* tadpoles (Güngördü et al. 2013). The algal EC_{50} of 0.025 mg/L (Greene and Baughman 1996) and the fish LC_{50} of 0.3 mg/L (Tonogai et al. 1982) were not considered for the aquatic CTV, given that the algae study may not represent the "true" effects of the test dyes on the organisms, due to possible light attenuation, and given that Güngördü et al. (2013) used a longer exposure duration (i.e., more sensitive) than Tonogai et al. (1982). The aquatic PNEC was then derived by dividing the CTV value (0.35 mg/L) by an assessment factor of 10 (to account for variability in interspecies and intraspecies sensitivity and to extrapolate from median lethal effects to no effects). Therefore, a PNEC of 0.035 mg/L was calculated for the Azo Basic Dyes.

6.2.2 Derivation of the predicted no-effect concentration (PNEC) for other environmental compartments

Due to the general lack of empirical studies found on the toxicity of Azo Basic Dyes to soil- or sediment-dwelling organisms, no soil or sediment PNECs were calculated for this assessment.

6.2.3 Ecological effects summary

Based on lines of evidence involving empirical and read-across aquatic ecotoxicity data and the low CTV value selected, Azo Basic Dyes may be expected to cause harm to aquatic organisms at low to moderate concentrations (i.e., LC_{50}s are < 10 mg/L). Based on limited empirical soil ecotoxicity data, Azo Basic Dyes are not likely to cause harm to soil-dwelling organisms at low concentrations.

6.3 Ecological Exposure Assessment

6.3.1 Releases to the environment

As no data on measured environmental concentrations (in water, soil or sediment) of the Azo Basic Dyes in Canada have been identified, environmental concentrations were estimated from available information.

Anthropogenic releases of a substance to the environment depend upon various losses that occur during the manufacture, industrial, consumer or commercial[4] use and disposal of a substance. In order to estimate releases to the environment occurring at different stages of the life cycle of the Azo Basic Dyes, Environment Canada compiled information on the relevant sectors and product lines as well as emission factors[5] to wastewater, land and air in order to identify the life cycle stages that are the largest contributors to environmental concentrations. Recycling activities and transfer to waste disposal sites (landfill, incineration) were also considered. However, releases to the environment from disposal were not quantitatively accounted for unless reliable specific information on the rate of (or potential for) release from landfills and incinerators was available.

In general, wastewater is a common point of entry of a substance into water through wastewater treatment system effluent and a potential point of entry into soil through the subsequent land application of biosolids. This information is used to further develop exposure scenarios to estimate resulting environmental concentrations.

6.3.2 Identification of important exposure scenarios

Factors relevant to the life cycle stages of these substances have been considered, uncertainties have been recognized and assumptions have been made, subject to the availability of information. Exposure scenarios for the uses or media of concern have been developed, including the determination of applicable predicted environmental concentrations (PECs).

The Azo Basic Dyes are not manufactured in Canada, according to the data collected from a 2011 regulatory survey for aromatic azo and benzidine-based substances (Canada 2011) and a 2009 Domestic Substances List Inventory Update (Canada

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[4] Commercial use is the use of a chemical substance, or the use of a mixture, product or manufactured item containing a chemical substance, in a commercial enterprise providing saleable goods or services.
[5] An emission factor is generally expressed as the fraction of a substance released to a given medium, such as wastewater, land or air, during a life cycle stage, such as manufacture, processing, industrial application or commercial/consumer use. Sources of emission factors include emission scenario documents developed under the auspices of the OECD, data reported to Environment Canada's National Pollutant Release Inventory, industry-generated data, monitoring data, etc.

2009a). Six were imported and used in the production of the following products categories derived from the uses identified in Table 4.1:

- Paper (dyeing and deinking)
- Textiles
- Pharmaceuticals
- Printing inks

Based on this use pattern, the following four scenarios of relevance to the ecological assessment are identified as the important potential sources of environmental releases:

- Pharmaceutical production
- Chemical formulation
- Paper dyeing
- Textile dyeing

Several additional scenarios are associated with the products indicated above, such as deinking, printing and printing ink manufacture and consumer use of textiles and pharmaceuticals. No quantitative exposure characterization is provided for the following reasons:

- *Deinking relating to paper*: The dyes contained in recycled paper are not readily released from paper fibre to process water during deinking and are degraded during the subsequent bleaching of the deinked pulp (Hannuksela and Rosencrance 2008; Huber and Carré 2012). The release of the dyes from deinking facilities is expected to be negligible.
- *Printing and printing ink manufacture (both relating to printing inks)*: Water release is not expected from printing facilities or printing ink manufacture facilities according to US EPA (1994). This was confirmed by one printing ink manufacture facility (personal communication, email from the company to Environment Canada, dated April 9, 2013; unreferenced).
- *Consumer use of textiles and pharmaceuticals*: Since the consumer use of textiles and pharmaceuticals is more dispersive than their industrial production, the environmental concentrations resulting from such use are expected to be lower than those from industrial production facilities.

6.3.3 Derivation of predicted environmental concentrations (PECs)

The water column is considered to be an important environmental compartment for the presence of the Azo Basic Dyes after they are discharged from industrial operations and then released to receiving water via industrial and/or publically-owned wastewater treatment systems. These dyes have moderate to high water solubilities, in the range of 0.1–340 g/L, and are therefore expected to be present in the water column upon release to surface water. The organic carbon–water partition coefficients of the Azo Basic Dyes were estimated to be moderate to high, with log K_{oc} values in the range of 3–5 (estimated). The partitioning to sediment or removal by sludge sorption in industrial or publically-owned wastewater treatment systems is therefore expected to occur to a certain extent. Nevertheless, the water column is still an important compartment, and

therefore water is selected as the primary medium for PEC calculations. PECs in soil resulting from biosolids land application and those in sediment resulting from water-to-sediment partitioning were not calculated.

Each of the four scenarios identified is generic in nature and consists of multiple facilities located across various sites. These facilities are identified as the industrial users of dyes from various sources, including survey data. They include facilities involved with the basic dyes as well as facilities involved with other dyes. The inclusion of the latter in each scenario is intended to account for the variability within the entire sector (not only for the period for which the survey data were obtained), the uncertainty associated with incomplete data and the likelihood of the switchover from other dyes to the basic dyes.

The aquatic PECs for the four scenarios or sectors are estimated for receiving water near discharge points. The method used for the estimation focuses on each sector as a whole and considers all actual and possible values for each variable that is known to vary within a certain range. As a result of this method, the aquatic PECs are derived as a probabilistic distribution. For a given sector, a percentile PEC provides an estimate for the percentage of PECs that are below the percentile PEC across the entire sector. For example, the 95th percentile PEC for the paper dyeing scenario is estimated as 4.84 µg/L in receiving water near discharge points, and 95% of the PECs from all paper dyeing sites would be below this value (see Table 6-4 below).

Note that the daily use quantity and emission factor for the paper dyeing scenario are based on data for dyes used in papermaking. For the chemical formulation and pharmaceutical production scenarios, values of the two parameters are not available for these specific scenarios, and generic values for the chemical industry found in the literature are used.

For textile dyeing, chemical formulation and pharmaceutical production, the equation used for the aquatic PEC calculations is given as:

$$PEC = (Q \times E \times (1-R) \times 10^9)/V$$

where:

- PEC: aquatic predicted environmental concentration, µg/L
- Q: daily use quantity of Azo Basic Dyes at a facility, kg/day
- E: emission factor of Azo Basic Dyes to process wastewater prior to any wastewater treatment, %
- R: removal of Azo Basic Dyes by industrial and/or publically-owned wastewater treatment systems, %
- V: daily dilution water volume near the discharge point of an industrial or publically-owned wastewater treatment system, L/d
- 10^9: conversion factor from kg to µg

For paper dyeing, the equation used for the aquatic PEC calculation is given as:

$$PEC = (A \times E \times (1-R) \times 10^9)/(F \times B \times D)$$

where:

PEC: aquatic predicted environmental concentration, µg/L
A: use rate of Azo Basic Dyes, kg of dyes per tonne of paper produced, or kg/t
E: emission factor of Azo Basic Dyes to process wastewater prior to any wastewater treatment, %
R: removal of Azo Basic Dyes by industrial and/or publically-owned wastewater treatment systems, %
F: mill effluent generation rate, L/t of paper
B: publically-owned wastewater dilution factor = publically-owned wastewater flow/mill effluent flow (B = 1 for direct discharge and B > 1 for indirect discharge)
D: receiving river dilution factor
10^9: conversion factor from kg to µg

For a given sector/scenario, the daily use quantity (Q) or use rate (A) and emission factor (E) are each determined to be in a certain range that is intended to be applicable to all facilities within the sector as well as to all possible operating conditions at a single facility. The removal by wastewater treatment systems (R) is a result of pollution mitigation on-site, off-site or both before the treated wastewater is released to the aquatic environment. The daily dilution water volume (V) or the per tonne dilution water volume (F × B × D) is derived based on several parameters, including daily wastewater volume from an industrial facility, wastewater flow from a publically-owned wastewater treatment system and the dilution factor of the receiving water near the discharge point of an industrial or publically-owned wastewater treatment system.

The removal by wastewater treatment (R) and the daily or per tonne dilution water volume (V or F × B × D) are characteristic of a site that has a certain level of mitigation for the substances present in wastewater and a certain degree of dilution for the substances released to the aquatic environment. In general, a sector/scenario consists of multiple sites, and the removal and the daily dilution water volume are determined as discrete distributions (sets of data points).

Table 6-3 provides a summary of the ranges of parameter values determined for each of the four sectors/scenarios identified.

Table 6-3: Parameter values used in aquatic PEC probabilistic distribution calculations

Parameter	Chemical formulation	Paper dyeing	Textile dyeing	Pharmaceutical production

Parameter	Chemical formulation	Paper dyeing	Textile dyeing	Pharmaceutical production
Number of sites	3	15	24	2
Number of facilities	3	17	42	2
Dye daily use quantity (kg/d)	5–650	N/A	9.1–36.3	5–100
Dye use rate (kg/t)	N/A	0.0031–0.031	N/A	N/A
Emission factor to wastewater (%)	0.3–2	2–10	0–4	0.3–2
Removal by wastewater treatment (%)	46.4	53.1–74.9	30.8–46.4	30.8–33.4
Daily dilution water volume (million L/d)	298–4970	N/A	7.4–3934	4.0–27 860
Per tonne dilution water volume (million L/t)	N/A	0.06–64	N/A	N/A

Abbreviation: N/A, not applicable

Crystal Ball, a commercial software program, is used to derive an aquatic PEC probabilistic distribution from the equation and the range of values determined for each parameter. In principle, a vast number of PECs are calculated by varying each parameter within the determined range (20 000–100 000 data points used for each range). These PECs represent different levels of exposure resulting from different conditions across all sites within a sector/scenario. The PECs derived are then sorted by their magnitudes and arranged as a function of the cumulative percentage or probability of occurrence (i.e., a probabilistic distribution).

The four probabilistic distributions are presented in Table 6-4. The lower PEC values in Table 6-4 correspond to lower values for daily use quantity or use rate and emission factor and higher values for removal and daily or per tonne dilution water volume. The conditions that result in higher PEC values are the reverse.

Table 6-4 shows that the probability of the PECs being below the PNEC (35 µg/L) is 95% or more for each of the four scenarios. This high probability indicates that the chance of exceeding the PNEC is unlikely when the conditions of facilities and their related sites fall within the ranges given in Table 6-3. The four probabilistic distributions presented in Table 6-4 are closely related to those conditions.

Table 6-4: Aquatic PECs for Azo Basic Dyes for four key industrial exposure scenarios

Percentile PEC	Pharmaceutical production (µg/L)	Textile dyeing (µg/L)	Chemical formulation (µg/L)	Paper dyeing (µg/L)
0th	0.03	0.00	0.00	0.02
5th	0.21	0.02	0.10	0.11

Percentile PEC	Pharmaceutical production (µg/L)	Textile dyeing (µg/L)	Chemical formulation (µg/L)	Paper dyeing (µg/L)
10th	0.34	0.04	0.18	0.17
25th	0.71	0.11	0.49	0.35
50th	1.52	0.43	2.30	0.73
75th	3.16	1.35	6.57	1.45
90th	7.28	3.56	11.1	2.81
95th	12.8	8.28	14.1	4.84
100th	154.8	46.1	22.7	19.2

6.4 Characterization of Ecological Risk

The approach taken in this ecological screening assessment was to examine various supporting information and develop conclusions based on a weight of evidence approach and using precaution, as required under CEPA 1999. Lines of evidence considered include information on physical and chemical properties, environmental fate, ecotoxicity and sources of the substances, as well as results from risk analyses, which are outlined below.

6.4.1 Aquatic probabilistic risk analysis

Risk analyses compare the PECs with the appropriate PNEC values in order to evaluate potential risks.

For the aquatic environment, a PNEC of 35 µg/L derived for Azo Basic Dyes (see Section 6.2 above) was compared with the PECs outlined in the previous section (see Table 6-4 in Section 6.3.3 above) for sites using Azo Basic Dyes for chemical formulation, paper dyeing, textile dyeing, and production of pharmaceuticals.

The probability of the PECs falling below the PNEC for all scenarios is high, indicating that the chance of exceeding the PNEC is very low (less than 5% for chemical formulation and unlikely for pharmaceutical production and textile dyeing) when the conditions of facilities and their related sites fall within the ranges given in Table 6-3.

6.4.2 Soil risk analysis

No risk analysis was performed for other compartments, because data were insufficient for determining a soil or sediment PNEC. Also, no PEC was determined, as no monitoring data were available and the substances are not within the domain of applicability of the exposure model for equilibrium partitioning.

6.4.3 Consideration of lines of evidence and conclusion of ecological risk characterization

Lines of evidence considered include results from conservative risk calculations, as well as information on persistence, bioaccumulation, ecological effects, sources and fate of the substances and their presence and distribution in the environment. Various lines of evidence from Azo Basic Dyes subset are summarized below, along with relevant uncertainties, leading to overall conclusions.

Azo Basic Dyes are anthropogenically produced and are not expected to occur naturally in the environment. No data concerning concentrations of these substances in the Canadian environment have been identified. Azo Basic Dyes are complex cationic molecules that have moderate to high water solubility and are expected to dissociate at environmentally relevant pH levels. Substances were divided into seven eco-subsets with respect to their physical, chemical and ecotoxicity properties. This grouping scheme permitted the use of analogues to fill in data gaps. Subsequent analysis of the physical and chemical properties, as well as the structural similarity of the Azo Basic Dyes, allowed for grouping for the analysis of environmental fate.

Due to their moderate to high water solubility and affinity for oppositely charged organic particles, Azo Basic Dyes are expected to be found in water, sediment and soil. Given their very low vapour pressures they are unlikely to stay in air if released to this compartment. Therefore, long-range atmospheric transport is not anticipated to be of concern. Given their hydrophilicity and charged character, Azo Basic Dyes have low experimental log K_{ow} values (< 2.03).

These dyes are not likely to bioconcentrate in aquatic organisms, based on their moderate molar weights (> 250 g/mol), relatively large minimum and maximum cross-sectional diameters and low log K_{ow} values, which suggest slow uptake potential. Bioaccumulation resulting from exposures of organisms to these substances in soil and sediment is not well understood due to limited data, in large part due to the moderate to high water solubility of these substances.

According to modelled data, Azo Basic Dyes are expected to biodegrade very slowly in aerobic environments and are therefore considered to be persistent in water, sediment and soil. However, Azo Basic Dyes may degrade and transform to certain aromatic amines in anaerobic environments.

Based on lines of evidence involving empirical aquatic ecotoxicity data for Azo Basic Dyes and analogues, it is concluded that Azo Basic Dyes may be hazardous to aquatic organisms at low concentrations (i.e., 0.3–13 mg/L). Toxicity data are limited for the terrestrial environment and unavailable for sediment-dwelling organisms.

A conservative exposure analysis of chemical formulation, paper dyeing, textile dyeing and pharmaceutical production processes was done because those sectors were anticipated to present the highest potential ecological risk related to industrial releases

to the environment for these substances. Using a probabilistic approach, the PECs were compared with the PNEC for water. The probability that the PECs of Azo Basic Dyes exceeded the PNEC was very low.

Considering all available lines of evidence presented in this screening assessment, there is low risk of harm to organisms and the broader integrity of the environment from Azo Basic Dyes is low. It is concluded that the 33 Azo Basic Dyes in this assessment do not meet the criteria under paragraph 64(*a*) or 64(*b*) of CEPA 1999, as they are not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

6.4.4 Uncertainties

In general, the Azo Basic Dyes subgroup addressed in this report were limited in the availability of ecological data. As a result, a read-across approach using data from selected analogues was the best alternative to estimate physical and chemical properties. This introduces some uncertainties, as there is still some degree of structural variation between the substances assessed and the analogues.

Additional long-term (chronic) toxicity data would be beneficial in evaluating these substances due to the fact that they are predicted to be persistent in the environment, but available documentation is scarce. The use of assessment factors in determining a PNEC is intended to address this uncertainty. While water was found to be the key medium of interest, soil and sediment also hold some importance due to potential adsorption and electrostatic interactions. Therefore, the limited available effects data for Azo Basic Dyes in soil and sediment are a source of uncertainty.

The lack of measured environmental concentrations of these substances (e.g., monitoring data) in Canada resulted in the need to evaluate risk based on predicted concentrations in water near industrial point sources. Conservative assumptions were made when using models to estimate concentrations in receiving water bodies.
Given the use of some of these substances in other countries, it is possible that they may enter the Canadian market as components of manufactured items and/or consumer products. However, it is anticipated that the proportions of these substances released to the various environmental media would not be significantly different from those estimated here, given the conservative assumptions used in the exposure analysis.

7. Potential to Cause Harm to Human Health

With respect to human health, the current screening assessment addresses 32 substances in the Azo Basic Dyes subgroup. NDTHPM, which was previously assessed under the Chemicals Management Plan, is considered only to inform the human health effects assessment. In addition, the current assessment focuses on substances with identified sources of exposure for the general population. For the purposes of human health assessment, those substances where exposure to the general population was expected were placed in subgroups based on structural similarity, as outlined in Table 7-1, below.

Table 7-1: Subsets of the 12 Azo Basic Dyes for which exposure of the general population of Canada is expected

Subset	C.I. name or CAS RN
A	Phenazopyridine hydrochloride, Basic Orange 2 and related salts[a] 63681-54-9 and 75660-25-2, Basic Brown 1
B	14408-20-9, 36986-04-6 and 59709-10-3
C	68929-07-7 and 69852-41-1
N/A	93783-70-1
N/A	52769-39-8

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index; N/A, not applicable as unable to place into one of the existing subsets.

[a] Basic Orange 2 (hydrochloride salt), 63681-54-9 (dodecyl-benzenesulfonate salt) and 75660-25-2 (acetate salt) are all salts of the same organic cation, 4-(phenylazo)-1,3-benzenediamine (known commonly as the free base form CI Solvent Orange 3, which as been previously evaluated in the assessment of Azo Solvent Dyes (Environment Canada, Health Canada 2015a).

7.1 Exposure Assessment

7.1.1 Environmental media

Empirical data on concentrations of the Azo Basic Dyes in environmental media in Canada or elsewhere were not identified. As described in Section 4.1, five Azo Basic Dyes (Basic Brown 1, Phenazopyridine hydrochloride and CAS RNs 52769-39-8, 63681-54-9, and 75660-25-2) were identified as being imported into Canada in quantities that are not expected to create a significant source of environmental exposure (NDTHPM was not assessed in the current screening assessment as it was previously assessed under the Challenge Initiative of the CMP and the conclusion under paragraph 64*(c)* is not being updated in this assessment) (Environment Canada, Health Canada 2010). Additionally, information pertaining to the use volumes of seven Azo Basic Dyes identified as being used in Canada was not available. Due to the very low volatility and limited commercial quantities of Azo Basic Dyes, environmental media are

not considered to be a significant source of exposure to these substances for the general population in Canada.

The potential for indirect exposure of the general population of Canada to phenazopyridine hydrochloride from its use as a pharmaceutical product is expected to be minimal due to its low quantity in use in Canada (< 0.10 kg per year) reported in 2011 and 2012 (MIDAS 2013).

7.1.2 Consumer products and cosmetics

A number of exposure scenarios are considered to be relevant to general population exposure in Canada, including scenarios from use of or contact with hair dyes, paper, textiles and ballpoint pen ink. Where substance-specific information was available, exposure estimates were derived for each substance. Otherwise, additional information from previous assessments (NDTHPM, Azo Benzidine Dyes) or the generic default parameters were used to characterize exposure during use of, or contact with, these products (refer to Appendix B). Estimated exposures are summarized in Table 7-2 and and details are available in Appendix B.

Subset A (Phenazopyridine hydrochloride, Basic Orange 2 and related salts (CAS RNs 63681-54-9 and 75660-25-2), and Basic Brown 1, and)

Three of the five substances in Subset A were considered to have potential exposure based on available data; exposure to Basic Orange 2 was quantified for hair dyes and pen ink while potential exposure from use in paper was qualitatively characterized for Basic Brown 1 and CAS RN 75660-25-2. For two substances in Subset A, phenazopyridine hydrochloride and CAS RN 63681-54-9, exposures to the general population from consumer products is not expected. More details are provided in the following sections.

Dermal exposure to Basic Orange 2 in hair dyes is estimated and summarized in Table 7-2 (refer to Appendix B for further details). The use of Basic Orange 2 in hair dyes is very limited relative to the wide range of hair dye products available to consumers in Canada. Inhalation exposure is expected to be negligible due to the very low vapour pressure of these substances. Exposure via inhalation to droplets of spray products is considered to be minor relative to dermal and oral exposures.

Basic Orange 2 was also identified as an ingredient of ink of ballpoint pens available in Canada (Smoothline Writing Instruments, 2010). Dermal and incidental oral exposure was estimated and summarized in Table 7-2 (refer to Appendix B for further details).

Table 7-2: Generic exposure to one of the five Azo Basic Dyes in subset A

Consumer or cosmetic product	Age group	Substance	Concentration range (% w/w)	Oral exposure per event (mg/kg-bw)	Dermal exposure per event (mg/kg-bw)
Hair dye semi-permanent	Adult	Basic Orange 2	0.3–1[a]	—	0.15–0.49
Hair dye permanent	Adult	Basic Orange 2	0.3–1[a]	—	0.42–1.4
Writing Ink	Toddler	Basic Orange 2	1.5	0.002[b]	0.002[b]

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; kg-bw, kilograms of body weight; w/w, weight per weight

[a] Notifications submitted under the *Cosmetic Regulations* to Health Canada (personal communications, emails from Consumer Product Safety Directorate [Health Canada] to Existing Substances Risk Assessment Bureau [Health Canada], dated 2011 and 2013; unreferenced).

[b] The exposure from writing ink to toddlers is considered an incidental scenario

CAS RN 75660-25-2 was reported to be used in paper in a section s71 survey and mouthing of paper products is considered a potential worst-case sentinel exposure for this type of use. Although toddlers may have incidental oral exposure resulting from mouthing of paper, there is uncertainty regarding in the actual amount ingested and the dye fastness to the paper following oral ingestion. Azo Basic Dyes have a high affinity for unbleached pulps and wood pulps in paper (Hunger 2003). In the assessment of NDTHPM (a dye included in the Azo Basic Dye subgroup but previously assessed under the Challenge Initiative under the CMP), oral exposure from incidental ingestion of paper was estimated to be 0.6 mg/kg bw per-event for children from 0.5-4 years of age (Environment Canada, Health Canada 2010); however assumptions used to derive this estimate were very conservative. Overall, mouthing of paper by toddlers is considered incidental and low. Although Basic Brown 1 was also identified as being used in paper products, it is not expected to be present in products available to children based on follow-up as a result of information submitted in the section 71 survey (Environment Canada 2012); therefore, oral exposure to toddlers from mouthing paper is not expected for this substance. Overall, use of Basic Brown 1 and CAS RN 75660-25-2 in paper is not expected to result in a significant source of exposure to the general population.

For two of the five remaining substances in Subset A (phenazopyridine hydrochloride and CAS RN 63681-54-9), exposures to the general population are not expected.

Drugs containing phenazopyridine hydrochloride as an ingredient were previously assessed under the *Food and Drugs Act* (Canada 1985) with respect to their safety, effectiveness and quality. Based on available information, no other potential consumer product uses were identified for phenazopyridine hydrochloride. Thus, non-drug exposures are not expected for this substance.

Although CAS-RN 63681-54-9 was reported as being imported for use in Canada, it was indicated in response to a section 71 survey that the products are no longer

present in the Canadian market, therefore exposure to this substance is not expected (Canada 2009a, 2012a).

Subset B (CAS RNs 14408-20-9, 36986-04-6 and 59709-10-3), Subset C (CAS RNs 68929-07-7 and 69852-41-1), CAS RN 52769-39-8 and CAS RN 93783-70-1

Although CAS RN 52769-39-8 was identified to be used as an ink, toner and/or colourant, as indicated in Section 4.2, the end-use product was identified as a textile dye. Therefore, for this substance, exposure of the general population of Canada was characterized based on contact with textiles. The other six substances (CAS RNs 14408-20-9, 36986-04-6, 59709-10-3, 68929-07-7, 69852-41-1 and 93783-70-1) did not have any information on their end-use products, although they had been identified as being used in Canada (personal communication, email from ETAD to Environment Canada, dated 2010; unreferenced). In the absence of substance-specific information on their potential uses, and considering the known generic use of basic dyes in textile and paper dyeing (Hunger 2003; Canada. 2012a), exposure estimates based on their use as dyes in textiles were derived. The potential exposure from paper is considered incidental and low, as noted above in the Subset A discussion. Exposure factors were the same for CAS 52769-39-8 and the other six substances, and therefore the exposure estimates presented in Table 7-3 are applicable to all seven substances, including CAS 52769-39-8, for textiles. These estimates are based on generic scenarios, incorporating an adjustment factor of 10% for detection of non-EU22 aromatic amines in textiles, which is generally less than 10% for associated dyes (Environment Canada and Health Canada, 2014a).

Table 7-3: Generic exposure estimates for CAS RNs 14408-20-9, 36986-04-6, 52769-39-8, 59709-10-3, 68929-07-7, 69852-41-1 and 93783-70-1 considering probability that the dye is present in textiles (Environment Canada and Health Canada, 2014a).

Consumer or cosmetic product	Age group	Concentration range (% w/w)	Oral exposure per event (mg/kg-bw)	Oral exposure daily (mg/kg-bw per day)	Dermal exposure daily (mg/kg-bw per day)[b]
Textiles	Adult	1[a]	—	—	0.0026
Textiles	Infant	1[a]	—	2.7×10^{-5}	0.0040

Abbreviations: kg-bw, kilograms of body weight; w/w, weight per weight

[a] BfR (2007).
[b] Dermal absorption was conservatively assumed to be 100%

7.1.3 Uncertainty

Estimated exposure to Basic Orange 2 from hair dye is based on conservative assumptions (see Appendix B), including assuming 100% dermal absorption as a tier 1 default value, although the dermal uptake of these substances is considered to be

limited. There could, however, be increased absorption if azo bond cleavage occurred, releasing aromatic amines that could be more readily absorbed.

The exposure factors used in deriving estimates are based on generic assumptions for dyes, but are not specific to Azo Basic Dyes. Similarly, uncertainty exists with respect to the exposure from those dyes identified for use by ETAD, since the potential end-use products are unknown. Exposure to Azo Basic Dyes that may be present in textiles and paper products (CAS RNs 14408-20-9, 36986-04-6, 59709-10-3, 68929-07-7, 69852-41-1, 93783-70-1 and 52769-39-8) was conservatively estimated based on general uses of Azo Basic Dyes, and generic estimates are not specific to Azo Basic Dyes.

With regard to the oral exposure scenario for incidental paper ingestion by toddlers, there is uncertainty regarding the amount of paper ingested by a toddler during a mouthing event and dye-bioavailability from treated paper.

7.2 Health Effects Assessment

The focus of the health effects assessment of Azo Basic Dyes was on 12 substances for which exposure of the general population of Canada is expected (see Section 7.1). NDTHPM was previously assessed under the Chemicals Management Plan (Environment Canada, Health Canada 2010). This substance was concluded to not meet the criteria under section 64*(c)* of CEPA 1999, however it was identified to have health effects of concern based on the potential to undergo azo reductive cleavage to the carcinogenic EU22 aromatic amine, *p*-aminoazobenzene. No significant new information relevant to the health conclusions was identified for NDTHPM, therefore the previous health conclusion has not been updated for this substance.

For the 20 substances for which exposure of the general population of Canada is not expected, the health effects characterization of these substances and their azo cleavage metabolites focused primarily on available data for carcinogenicity and genotoxicity.

Carcinogenicity and genotoxicity are generally considered to be the critical health effects of potential concern for the Aromatic Azo and Benzidine-based Substance Grouping (Environment Canada and Health Canada 2013). One of the primary mechanisms by which aromatic azo substances exert their toxicity involves the reductive cleavage of the azo bonds and the subsequent release of the free aromatic amines. These aromatic amines can be converted to reactive electrophilic intermediates through metabolic oxidation (Environment Canada and Health Canada 2013). Based on information for two Azo Basic Dyes and read-across considerations for the remaining 10 substances (see Section 7.2.1), the Azo Basic Dyes are considered to have some potential to undergo reductive cleavage. Therefore, the health effects of the Azo Basic Dyes were assessed in part by examining their ability to undergo reductive cleavage and the hazard potential of the corresponding aromatic amine metabolites.

In addition, azo substances that are more lipophilic may be absorbed from the gastrointestinal tract as the parent substance with the azo bond intact as is observed for several azo solvent dyes (Environment Canada, Health Canada 2015a). Since azo basic dyes of the weak base type (i.e. Subset A) demonstrate pH-dependent ionization (see Section 3. Physical and Chemical Properties), these substances tend to be uncharged and lipophilic at neutral pH and therefore may also be absorbed as the parent substance with azo bond intact. Some Azo Basic Dyes are reported to be absorbed prior to azo bond cleavage (IARC 1980; Holahan et al, 2010) including one of the Azo Basic Dyes in this assessment, phenazopyridine hydrochloride, based on *in vivo* studies in humans and animal models (see Section 7.2.1). If the absorbed parent azo substance possesses one or more free aromatic amines, it can be readily metabolized to electrophilic intermediates without azo bond cleavage. *In vitro* data showed that the parent azo substance can be directly metabolized to electrophilic intermediates via the free aromatic amines on the parent substance (Sandhu and Chipman 1991b), and is considered to be the activation pathway for certain azo solvent dyes such as *p*-aminoazobenzene and Solvent Yellow 2 (Environment Canada, Health Canada 2015a). Therefore, in addition to the potential toxicity from aromatic amine metabolites of azo bond cleavage, direct oxidation of the free aromatic amine moiety on the parent azo may also be a potential activation pathway for some of the Azo Basic Dyes with this structural feature (i.e. substances in Subset A, see Section 7.2.2).

Overall, limited empirical data were identified on the health effects of these 12 Azo Basic Dyes. Due to the limited data available for these substances, toxicological data on similar substances were also considered to inform the health effects assessment. The subsets were derived to aid in read-across among similar substances and was based on chemical structure and physical-chemical properties, when available. As such, related salts were considered to be toxicologically equivalent and grouped together in the same subset (e.g. Basic Orange 2 and related salts CAS RNs 63681-54-9 and 75660-25-2 are grouped together in Subset A). Some of these similar substances were identified using the OECD QSAR Toolbox (2013), the C.I. International database (CII 2011) and literature searches via TOXLINE and SciFinder; using parameters considered relevant including dye application class, structural similarity (presence of azo bond(s), presence of aryl functional group), physical and chemical properties (molecular size and solubility) and mode of action (potential to release the same reactive azo reductive products).

7.2.1 Absorption, Metabolism, and Azo Bond Cleavage Potential

Limited data were identified on absorption, metabolism and azo cleavage potential for substances in the Azo Basic dyes assessment and were limited to several *in vivo* metabolism studies on a single substance, phenazopyridine hydrochloride, as well as *in vitro* studies examining azo bond cleavage potential for Basic Brown 1. This information is summarized below.

In Vivo **Data**

In vivo studies are considered to provide the most relevant data to characterize absorption, metabolism and azo bond cleavage of azo substances and this type of data was identified for only one of the Azo Basic Dyes from Subset A, phenazopyridine hydrochloride. Phenazopyridine hydrochloride is considered to be absorbed, then conjugated or reduced when administered orally to humans; the majority is eliminated in the urine (IARC 1980). Phenazopyridine hydrochloride is considered to be absorbed based on evidence from rats administered radiolabelled phenazopyridine hydrochloride via gavage at a dose of 100 mg/kg body weight (kg-bw) (Thomas et al. 1993).

Absorption of phenazopyridine hydrochloride was noted to be rapid from the gastrointestinal tract of rats, with subsequent distribution to the liver and kidneys. Bile was noted to be the major route of excretion, where a smaller percentage was excreted through urine (Thomas et al. 1993). The product monograph for pharmaceutical product containing phenazopyridine hydrochloride as active ingredient notes that discolouration of urine, feces, sclera or skin of humans is a possible side effect of taking this drug (Erfa Canada Inc. 2010).

Major metabolites of phenazopyridine hydrochloride eliminated in urine of humans 36 hours after oral administration were the unmodified or ring-hydroxylated parent phenazopyridine, as well as the azo cleavage product aniline and its hydroxylated and N-acetylated metabolites (*p*-aminophenol and *N*-acetyl-4-aminophenol (NAPA)) (Johnson and Chartrand 1976; Thomas et al. 1990).

Additional studies regarding metabolism and elimination of phenazopyridine hydrochloride were identified in other experimental animal species where the dominant excretion route and product (metabolite or conjugate) is different between species (Johnson and Chartrand 1976; Bailey et al. 1983; Thomas et al. 1993, 1990; Jurima-Romet et al. 1993). The major component of biliary excretion after oral administration of phenazopyridine hydrochloride in rats includes 2,6-diamino-3-(4-hydroxyphenylazo)pyridine (4′-OH-PAP), and a glucuronide conjugate (Thomas et al., 1993). Major urinary metabolites in rats, mice, rabbits or guinea pigs include NAPA, *p*-aminophenol, 2,6-diamino-5-hydroxy-3-(4-hydroxyphenylazo)pyridine (5,4′-diOH-PAP), 4′-OH-PAP and 5-OH-PAP (Johnson and Chartrand 1976; Bailey et al., 1983; Thomas et al., 1990, 1993). There are differences between species regarding the dominant route of elimination (Thomas et al., 1993, 1990), as well as the extent of azo bond cleavage, which is high in mice and guinea pigs, moderate in rats and low in humans exposed to phenazopyridine hydrochloride (Thomas et al. 1990). In summary, the available data on phenazopyiridine hydrochloride indicates that it is absorbed from the oral route as both the parent substance with azo bond intact, and also can undergo azo cleavage to aromatic amine metabolites.

Two *in vivo* studies on Basic Orange 2 provide only limited information on the metabolism of this substance. Briefly, it was found that the dye was excreted in the urine following oral dosing to rats (Cambel et al. 1954), and that reduction of the Basic Orange 2 depends on the digestive system and microflora when administered to dogs

(Sisley and Porcher 1911), and that when fed to mice a small amount was found bound to liver proteins (Piekarski and Marciszewski 1966). Despite this limited data, it is is expected that the absorption and azo cleavage of Basic Orange 2 and related salts CAS RNs 63681-54-9 and 75660-25-2 from the gastrointestinal tract will behave similarly to that observed for phenazopyridine hydrochloride, based on their structural similarlity, similar physical-chemical properties, and modelled pH-dependent ionization and solubility (see Section 3. Physical and Chemical Properties).

In Vitro Data

An *in vitro* metabolism study was available for Basic Brown 1 in which the dye was incubated with intestinal contents, feces and liver extracts from mammalian species (BRI 2013). In this study, the disappearance of the parent Basic Brown 1 from the incubation medium over the first 4–8 hours of a 24-hour incubation period is indicative of rapid reductive cleavage of the azo bond of this substance.

Positive results obtained from the Ames assay only when conducted under reductive conditions can also be considered to infer reductive cleavage potential when the parent azo is either negative or weakly mutagenic under standard conditions (Environment Canada and Health Canada 2013). CAS RN 75660-25-2 and CAS RN 68929-07-7 produced positive results in Salmonella typhimurium TA98 under reductive conditions (BioReliance 2012). Negative results in S. typhimurium TA100 under reductive conditions were observed for Basic Orange 2 and CAS RN 75660-25-2 and CAS RN 68929-07-7 (Sandhu and Chipman 1991b; BioReliance 2012). However, since both substances were also reported to be positive under standard non-reductive conditions in this study, it is likely that the parent limited information on azo cleavage potential can be inferred in strain TA 100 for these substances.

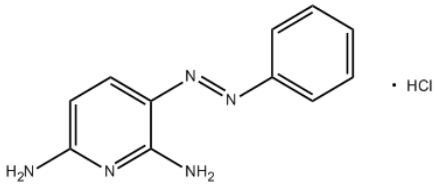
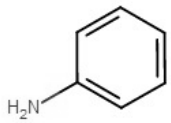
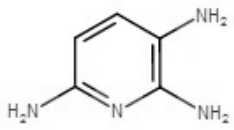
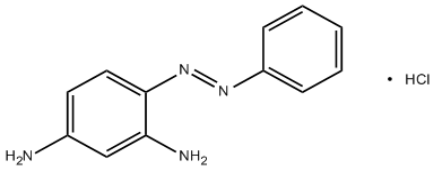
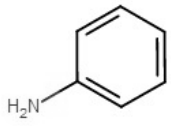
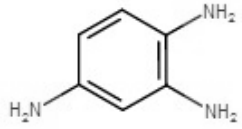
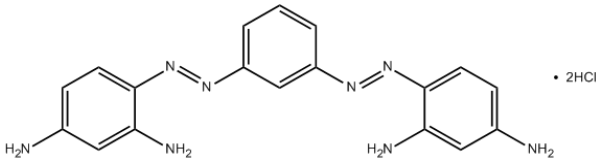
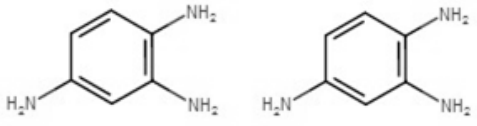
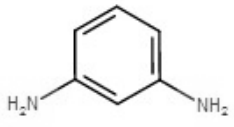
In summary, the available data on two substances from Subset A (phenazopyridine hydrochloride, Basic Brown 1) suggest these substances may be absorbed as the parent azo substance as well a potential to undergo azo bond cleavage from the oral route. In the absence of data on the remaining Azo Basic Dyes in this assessment, it is considered that a similar potential may also exist for these substances and is consistant with the body of knowledge avaible for most aromatic azo substances in general (Environment Canada, Health Canada 2013).

7.2.2 Health Effects Data for Subset A (Phenazopyridine hydrochloride, Basic Orange 2 and related salts CAS RNs 63681-54-9 and 75660-25-2, Basic Brown 1)

Substances in Subset A share several structural and chemical characteristics. These substances share at least one common azo bond cleavage metabolite (Table 7-4). Phenazopyridine and Basic Orange 2 (and related salts) are both monoazo substances containing the same aniline moiety joined by an azo bond to structurally similar aromatic polyamines: 2,3,6-triaminopyridine and 1,2,4-triaminobenzene, respectively. Basic Brown 1 is a disazo substance which also hosts two moieties of 1,2,4-triaminobenzene

azo bonded to 1,3-diaminobenzene. The health effects data of the Subset A substances are described in the following sections.

Table 7-4: Subset A substance structures and potential azo cleavage products

Parent substance	Potential azo bond cleavage products
phenazopyridine hydrochloride 	aniline (CAS RN 62-53-3)  2,3,6-triaminopyridine (CAS RN 4318-79-0) 
Basic Orange 2 and related salts[a] CAS RNs 63681-54-9 and 75660-25-2 	aniline (CAS RN 62-53-3)  1,2,4-triaminobenzene (CAS RN 615-71-4) 
Basic Brown 1 	1,2,4-triaminobenzene (CAS RN 615-71-4)  1,3-benzenediamine (CAS RN 108-45-2) 

[a] shown as the hydrochloride salt form (Basic Orange 2); for the related salts CAS RNs 63681-54-9 and 75660-25-2 the H.Cl salt would be replaced with dodecyl-benzene sulphonic acid and acetic acid respectively.

Phenazopyridine hydrochloride

Carcinogenicity and genotoxicity

Phenazopyridine hydrochloride, which is used as a urinary tract analgesic in prescription pharmaceuticals (see Section 4.2 Uses), has been reviewed and classified by the International Agency for Research on Cancer (IARC) as Group 2B (possibly carcinogenic to humans) (IARC 1975b, 1980, 1987a). In the Second and Twelfth Report on Carcinogens, the US National Toxicology Program (NTP) listed phenazopyridine hydrochloride as being “reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals” (NTP 1981, 2011). Both IARC and the NTP used the same cancer study (NCI 1978), noted in the previous section, as the basis for their conclusions on phenazopyridine hydrochloride.

In one epidemiological study, no significant excess cancer incidence was reported among 2214 patients treated with phenazopyridine hydrochloride and subsequently followed for a minimum of 3 years (Friedman and Ury 1980; IARC 1980). The IARC evaluation cited limitations of the study due to a lack of details on the age and sex distributions of the exposed subjects and the doses and durations of use of phenazopyridine. While a subsequent publication on the same cohort with an additional 12yrs follow-up time continue to report no association with cancer risk from phenazopyridine treatment (Van den Eeden and Friedman 1995; Habel and Friedman 2006), the limitations originally reported by IARC still apply. However, the lack of a clear association in this cohort provides evidence that this substance is not likely a potent carcinogen under the exposure conditions of the studied population.

A carcinogenicity study was conducted for phenazopyridine hydrochloride in F344 rats and B6C3F1 mice administered through the diet (NCI 1978). Groups of 35 animals per sex were treated with 3700 or 7500 ppm (180 and 415 mg/kg-bw per day for males; 228 and 613 mg/kg-bw per day for females) for 78 weeks followed by 26 or 27 weeks observation for rats. Mice were administered 600 or 1200 ppm (65 and 137 mg/kg-bw per day for males; 67 and 150 mg/kg-bw per day for females) for 80 weeks followed by 25 to 27 weeks of observation. Groups of 15 untreated rats and mice of each sex were used as matched controls. Sacrifice of rats was conducted at 104 to 105 weeks, and at 105-107 weeks for mice. Decreased body weight in rats and mice compared to control was found to be a dose-related effect, while mortality in rats and mice was not determined to be a dose-related trend (NCI 1978).

Observed carcinogenic effects in rats include a statistically significant dose-related trend for formation of adenoma and adenocarcinoma of the large intestine (colon, rectum) in male F344 rats, but not females (male: control, low, high = 0/14, 4/34, 8/35; female: control, low, high = 0/15, 3/33, 5/32). However, in comparison to the low incidence of adenoma and adenocarcinoma of the large intestine in historical control rats from this laboratory (0/260 in females; 1 polyp in 260 males), the NTP considered the large intestine tumours in this study to be related to phenazopyridine exposure. An observed

decrease in incidence of interstitial-cell tumours in the testes of high-dose male rats and fibroadenoma of mammary gland in females was determined to be due to increased incidence in the controls, and was not considered to be related to treatment with phenazopyridine hydrochloride (NCI 1978).

In female B6C3F1 mice, a statistically significant dose-related trend was reported for hepatocellular carcinoma (P=0.01) as well as combined hepatocellular adenoma or carcinoma (P=0.002) compared to controls. At the high-dose level in female mice, a statistically significant increase in combined hepatocellular adenoma or carcinoma was reported compared to control (control, low, high = 2/15, 11/34, 19/32). In male mice, there was no report of significant response when the same tumour types were considered alone or in combination (control, low, high = 2/15, 11/34, 19/32), nor was metastasis reported (NCI 1978). The study was considered “negative” for carcinogenicity of phenazopyridine in male mice.

Additional carcinogenicity studies in mice identified for phenazopyridine hydrochloride (Allen et al. 1957; Stoner et al. 1973) were not included in this screening assessment due to limitations (IARC 1975b, 1980), in addition to the routes of administration (intraperitoneal injection, implantation into the urinary bladder) which were not considered relevant to the exposure scenarios previously characterized.

Based on the studies presented above, phenazopyridine hydrochloride is considered to have potential for carcinogenicity due to the increased incidence of different tumour types in two species; adenoma or adenocarcinoma of the colon in rats and a combined incidence of hepatocellular adenoma and carcinoma in female mice (NCI 1978). And therefore, this data indicates a risk for carcinogenicity exists after long-term repeated exposure to phenazopyridine hydrochloride.

DNA damage *in vivo* was observed using the comet assay in the stomach, colon and liver of male ddY mice after a single oral exposure to phenazopyridine hydrochloride at 400 mg/kg-bw, deemed the maximum tolerated dose by the authors in a preliminary acute toxicity test (Tsuda et al. 2000). A clastogenic response was observed for phenazopyridine hydrochloride, due to an increase in micronucleus formation in the liver of rats and bone marrow of female CD-1 mice (Morita et al. 1997; Shirotori and Miyagawa 1997). Non-mammalian mutagenicity assays such as the sex-linked recessive lethal assay in *Drosophila* demonstrated equivocal results for phenazopyridine hydrochloride (Woodruff et al. 1985; Mason et al. 1992).

Results for *in vitro* mutagenicity were mixed, as phenazopyridine hydrochloride induced mutation at the thymidine kinase (*tk*) locus in mouse lymphoma cells (McGregor *et al.* 1991), but demonstrated equivocal results with metabolic activation or negative results with activation in various strains of *S. typhimurium* in the Ames assay (Mortelmans et al. 1986). Positive results were reported for DNA repair, as noted through unscheduled DNA synthesis in male rat hepatocytes when treated with phenazopyridine hydrochloride (Selden et al. 1994), as well as for clastogenicity through induction of

chromosomal aberrations and sister chromatid exchanges in Chinese hamster ovary cells (Galloway et al. 1987).

Based on the available information, phenazopyridine hydrochloride is considered to have carcinogenic potential following chronic exposure. There is some potential for genotoxicity, as observed through DNA damage and clastogenic responses *in vivo* and *in vitro*; however, there are mixed results for mutagenicity.

Other Health effects

Non-cancer effects were observed for phenazopyridine hydrochloride in the carcinogenicity study of F344 rats and B6C3F1 mice (NCI 1978). As summarized above, male and female rats (35 of each sex per treated group; 15 of each sex for control group) were administered phenazopyridine hydrochloride at a level of 3700 or 7500 ppm for rats (180 and 415 mg/kg-bw per day for males; 228 and 613 mg/kg-bw per day for females) for 78 weeks, then observed for an additional 26–27 weeks. Surviving animals were sacrificed at 104–105 weeks. The mean body weights were noted to be significantly lower in treatment groups; however, the mortality observed in the treatment groups was determined not to have dose-related trends. The study authors noted that many of the observed effects in the dosed and control groups may be related to age. The low-dose treatment level of 180 mg/kg-bw per day was determined to be the lowest-observed-adverse-effect level (LOAEL) for the male rats, due to an increased incidence of myocardial inflammation. Similar effects were observed in female rats at both dose levels. Incidence of thyroid C-cell hyperplasia and adenoma was combined due to difficulty in distinguishing between the lesion types based on similar appearance. In male rats, the combined incidence at the high dose may also be of concern. An increased incidence of bone marrow atrophy in female rats at the high-dose treatment level was also observed (NCI 1978).

Male and female mice were administered phenazopyridine hydrochloride at a level of 600 or 1200 ppm (65 and 137 mg/kg-bw per day for males; 67 and 150 mg/kg-bw per day for females) for 80 weeks, with an additional 25–27 weeks of observation. The remaining mice were sacrificed at 105–107 weeks. A significant decrease in mean body weight was noted, but the observed mortality was determined not to have dose-related trends. Similar to the rats treated in the same study, the authors noted that many of the observed effects in both control and treatment groups may be related to age (NCI 1978). A no-observed-effect level (NOEL) of 150 mg/kg-bw per day, the highest dose tested, was determined for mice.

Reproductive and short-term toxicity studies in rats and rabbits were identified for phenazopyridine hydrochloride administered with sulfacytine, a sulfonamide antibiotic (Erfa Canada Inc 2010); however, they were considered to be of limited utility due to the combination of substance being administered and the relevance of the observed effects to humans.

Postulated azo bond cleavage products

The postulated azo reductive cleavage products of phenazopyridine hydrochloride are aniline (CAS RN 62-53-3) and 2,3,6-triaminopyridine (CAS RN 4318-79-0). See Table 7-4 and also Appendix C (Tables C1 and C2).

Aniline was previously evaluated by Health Canada (2011a) and therefore only a brief description of the health effects follows. The genotoxicity of aniline in various *in vitro* or *in vivo* assays was mixed, but was generally observed only at high doses. In carcinogenicity studies, aniline induced a rare spectrum of tumours in the spleen of male Fischer 344 rats at very high doses at which substantial effects on the red blood cells and non-neoplastic splenotoxicity secondary to methemoglobinemia were also observed. Additional information on aniline can be found in Health Canada (2011a).

No classifications by other national or international agencies were identified for 2,3,6-triaminopyridine (CAS RN 4318-79-0) and no chronic toxicity data or genotoxicity data were identified for this substance. Limited available data suggestes this substance undergoes autooxidation *in vivo* to generate reactive oxygen species which may be responsible for the mytoxicity observed in rats exposed to this substance including necrosis of skeletal and cardiac muscle (Munday and Manns 1998).

The observation of increased hematopoiesis in the spleen of rats of both sexes exposed to phenazopyridine hydrochloride, while not dose-dependent (NCI 1978) is indicative of an aniline-type response, and evidence that some degree of azo bond cleavage had occurred. Furthermore, the myocardial inflammation in rats exposed to phenazopyridine hydrochloride (NCI 1978) is also consistent with the toxicity of the other azo cleavage metabolite, 2,3,6-triaminopyridine. However, the types of tumours observed following phenazopyridine exposure (i.e. colon in rats, liver in mice) are not typical of aniline exposure (spleen sarcomas in rats) indicating that aniline is not the primary contributor to the carcinogenicity of phenazopyridine. In the absence of chronic toxicity data on 2,3,6-triaminopyridine, the potential contribution of this substance to the carcinogenicity of phenezopyridine hydrochloridine is unknown.

Basic Orange 2 and related salts CAS RNs 63681-54-9 and 75660-25-2

Note: The free base form of Basic Orange 2 and related salts is 4-(phenylazo)-1,3-benzenediamine, commonly known as CI Solvent Orange 3 (CAS RN 495-54-5). These substances are expected for form the same chemical species *in vivo* and are considered to be toxicologically equivalent. Therefore the health effects data of all the substances are evaluated together. Solvent Orange 3 as been previously evaluated in the assessment of Azo Solvent Dyes (Environment Canada, Health Canada 2015a) and additional information on the health effects for these substances has also been reported in this previous assessment.

Basic Orange 2, under the name “chrysoidine”, has been reviewed by IARC and considered to be carcinogenic in mice based on limited evidence while the evidence for

carcinogenicity in humans was inadequate (Group 3 “not classifiable as to its carcinogenicity to humans”) (IARC 1975a, 1987b). In addition, Basic Orange 2 and related salts CAS RN 63681-54-9 and CAS RN 75660-25-2 have been classified as Category 2 mutagens (“suspected of causing genetic defects”) by the EU as a group (including CAS RN79234-33-6, 83968-67-6 and 84196-22-5) (European Commission 2009).

Several case reports and case-control studies of bladder cancer in amateur fishermen from the United Kingdom orally exposed to maggots dyed with Basic Orange 2 were reviewed by IARC (Searle and Teale 1982; Cartwright et al. 1983; Sole and Sorahan 1985) were considered as providing inadequate evidence for carcinogenicity in humans (IARC 1975a, 1987b).

Carcinogenicity studies of Basic Orange 2 were conducted in single experiments in mice and rats. Male and female C57BL mice (60 of each sex) were orally administered a low vitamin diet containing Basic Orange 2 at 2000 mg/kg daily (260 mg/kg-bw per day) for 13 months, then given control diet and observed until their natural deaths. Two additional groups (50 to 70 of each sex) acted as controls. Liver tumours were reported in mice after 10-11 months of observation (control = 1/89, 2/117; treatment = 75/104), in addition to metastasis of the lungs of three treated animals. Leukemias and reticulum-cell tumours were also reported (control = 9/89, 12/117; treatment = 28/104) (Albert 1956). Although issues have been raised regarding the age of the study, purity of the test substance and study methodology, IARC considered the study to provide evidence this substance “is carcinogenic in mice follawing its oral administration, producing liver-cell tumours, leukaemia and reticulum-cell sarcomas” (IARC 1975a, 1987b). In the dietary study in 10 rats fed Basic Orange 2 (concentrations of 1000 mg/kg in feed, equivalent to approximately 50 mg/kg-bw per day[6]) lasting up to 366 days, no tumours were reported (Maruya 1938), however IARC (1975a, 1987b) did not consider the study to have been adequately reported. With only a single dose group and lack of control animals, limited statistical power due to the small number of animals, and less than full life-time exposure and observation periodsthis study may not have fully explored the carcinogenic potential in rats, while it may provide evidence the substance is not a potent carcinogen under the conditions of the study Overall, while the available animal carcinogenicity data on Basic Orange 2 is limited, IARC considered this substance as being carcinogenic in mice (IARC 1975a, 1987b).

DNA damage *in vivo* (unscheduled DNA synthesis in rat hepatocytes) was observed when male F344 rats consumed Basic Orange 2 at doses up to 2000 mg/kg-bw (BASF 1991). At doses up to 300 mg/kg body weight, negative clastogenicity results were observed in the micronucleus assay in mice (BASF 1988). Similarily, non-mammalian mutagenicity assays, such as the sex-linked recessive lethal assay in *Drosophila*, negative results were obtained (Foureman et al. 1994).

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[6] Dose conversion in feed for rats: mg/kg-bw perday = ppm X 0.05 (Health Canada 1994)

Basic Orange 2 had mixed results for mutagenicity in various strains of *S. typhimurium* in the Ames assay (Garner and Nutman 1977; Sole and Chipman 1986; Zeiger et al. 1987; Sandhu and Chipman 1990, 1991a; NTP 1993). Positive *in vitro* results were reported for DNA repair (unscheduled DNA synthesis) in rat hepatocytes (Sandhu and Chipman 1990) and mitotic recombination in *Saccharomyces cerevisiae* (Zimina and Pavlenko 1990).

In vitro mutagenicity of CAS RN 75660-25-2 (the acetate salt) showed mixed results depending on the strain and test conditions. Positive results were obtained for CAS RN 75660-25-2 in a Prival-modified Ames assay when *S. typhimurium* TA100 was exposed under standard conditions (uninduced hamster S9) and in TA98 under standard and reductive (flavin mononucleotide) conditions. Negative results were obtained in TA100 under reductive conditions (BioReliance 2012).

No empirical health effects data were identified for CAS RN 63681-54-9 (the dodecyl-benzenesylfonate salt).

Overall, while based on limited available data, this substance is reported to be carcinogenic in mice (IARC 1975a, 1987b) and therefore Basic Orange 2 and related salts CAS RNs 63681-54-9 and 75660-25-2 are considered in this assessment to have some carcinogenic potential. Based on evaluation of the available data for genotoxicity, Basic Orange 2 and related salts are considered to be mutagenic, and may have the potential for DNA damage or impaired DNA repair, as observed in *in vivo* and *in vitro* assays.

Other Health effects

Male and female albino (Sprague-Dawley-Holtzman) rats (five of each sex per dose) were administered a single-dose level of 0.1% (total of 670 mg/rat or approximately 160 mg/kg-bw per day) Basic Orange 2 in drinking water daily for 21 days (Cambel et al. 1954). Animals were sacrificed after the treatment period. A statistically significant decrease in red blood cell counts (27% in females, 10% in males) and small decreases in percent hemoglobin levels in females were observed at the only dose tested (160 mg/kg-bw per day). Observed effects included significant increase in binucleated parietal cells of the stomach, as well as observation of pigmentation of the forestomach, glandular stomach secretions and small intestine (Cambel et al. 1954). This pigmentation is indicative of local exposure through the observed presence of the dye. The observed colour change of the urine is suggestive of systemic exposure after oral exposure. This study was limited in scope focussing on the blood and stomach, where the effects were not considered to be severe. An effect level of 160 mg/kg-bw per day was identified in this study based on the hematotoxicity and pigmentation.

Postulated Azo bond cleavage products

The postulated azo bond cleavage products for Basic Orange 2 and the related salts are aniline (CAS RN 62-53-3) and 1,2,4-triaminobenzene (CAS RN 615-71-4).

The health effects data for aniline were previously described in the preceding section on azo bond cleavage products for phenazopyridine and is not repeated here.

No classifications by other national or international agencies were identified for 1,2,4-triaminobenzene and no chronic toxicity data or genotoxicity data were identified for this substance. 1,2,4-triaminobenzene has been tested in a short-term study in rats (n=6-7) exposed orally to doses of 50, 60, 75, and 100 mg/kg-bw per day five days/week for 2 weeks. This substance was shown to very highly toxic with lethality observed in the 2 highest doses after the 3 doses. Animals in the lower doses showed extensive heart pigmentation and cardiac necrosis. The pigmentation is considered to be likely an insoluble oxidation product of 1,2,4-triaminobenzene and has also been observed in animals exposed to the dye Brown FK, of which 1,2,4-triaminobenzene is one of the metabolites (JECFA 1977).

The short-term study in rats exposed to Basic Orange 2 showed evidence of hematotoxicity (Cambel et al. 1954) consistent with that expected from aniline, suggesting some azo bond cleavage had occurred in this study. However, the types of tumours observed following chronic exposure to Basic Orange 2 exposure (i.e. liver in mice) is not typical of aniline exposure (spleen sarcomas in rats) indicating that the toxicity of the aniline metabolite alone is unsufficient to explain the carcinogenicity of Basic Orange 2. In the absence of chronic toxicity data on the other azo cleavage product 1,2,4-triaminobenzene, the potential contribution of this substance to the carcinogenicity of Basic Orange 2 is unknown.

Basic Brown 1

Basic Brown 1 has not been assessed by any national or international program or agency and no animal toxicity data were identified for this substance. Positive results in the Ames assay were observed in *S. typhimurium* TA98 with and without activation (Matsushima et al. 1978; NTP 1992a), but mixed results were reported when tested in TA100 (Sole and Chipman 1986; NTP 1992a).

The postulated azo bond cleavage products of Basic Brown 1 are 1,2,4-triaminobenzene (described in previous section for Basic Orange 2) and 1,3-benzenediamine (CAS RN 108-45-2). 1,3-benzenediamine has been evaluated in the assessment of Certain Aromatic Amines (Environment Canada, Health Canada 2015b) and details of the health effects data on this substance can be found in that assessment. Briefly, 1,3-benzenediamine has been classified by the EU under the Harmonized Classification as a Category 2 mutagen (suspected of causing genetic defects) (European Commission 2009) and has also been classified as a Group 3 carcinogen (not classifiable as to its carcinogenicity to humans) by IARC (1987c).

Rationale for read-across from phenazopyridine hydrochloride to Basic Orange 2 and related salts

Overall, available data indicate that phenazopyridine hydrochloride has potential for carcinogenicity, as male and female F344 rats administered this substance in the diet for 78 weeks had an increased incidence of adenoma or adenocarcinoma of the colon, which was considered statistically significant at the highest dose level tested (i.e., 7500 ppm) when compared with the historical controls. In female B6C3F1 mice administered this substance through the diet for 80 weeks, the combined incidence of hepatocellular adenoma and carcinoma was found to be statistically significant when compared with study controls (NCI 1978). Based on limited data, Basic Orange 2 was also considered to be carcinogenic in the liver of female mice (Albert 1956; IARC 1975a, 1987b).

For phenazopyridine hydrochloride and Basic Orange 2, the observed tumours were different from that expected from their common azo cleavage metabolite aniline (spleen sarcomas). The observed carcinogenicity of phenazopyridine hydrochloride and Basic Orange 2 may be potentially explained by their other respective azo cleavage products: 2,3,6-triaminopyridine and 1,2,4-triaminobenzene. While both 1,2,4-triaminobenzene and 2,3,6-triaminopyridine have been suggested to potentially induce oxidative stress and are associated with muscle and/or cardiac toxicity (JECFA 1977; Munday and Fowke 1994; Munday 1986, 1987), a lack of chronic toxicity data on both azo cleavage metabolites precludes determination of their carcinogenic potential. Since both phenazopyridine hydrochloride and Basic Orange 2 are likely absorbed orally as the parent dye with intact azo bond, the free amine moieties of these substances may also be directly oxidized to reactive intermediates. This activation pathway is known to exist for other structurally-related monoazo solvent dyes *p*-aminoazobenzene and Solvent Yellow 2 both of which also contain an aniline moiety azo bonded to an aromatic ring bearing a free amine and dimethyl amine respectively (Environment Canada, Health Canada 2015a). For both *p*-aminoazobenzene and Solvent Yellow 2, the tumours induced are different from that expected from their corresponding azo cleavage products indicating the carcinogenicity was not due to azo bond cleavage, but rather the activation of the parent substance. Therefore, it is reasonable to assume the carcinogenicity of both phenazopyridine hydrochloride and Basic Orange 2 may similarly be due to activation of the free amine moieties of the absorbed parent substances.

While the carcinonogenicity data for Basic Orange 2 is more limited relative to that for phenazopyridine hydrochloride, given these substances similar structures including free aromatic amine moieties, physical-chemical properties, expected absorption/ metabolism, and their azo cleavage metabolites both common (aniline) and closely related polyamineted (1,2,4-triaminobenzene and 2,3,6-triaminopyridine), and the observation of liver tumours in mice for both substances, together supports a read-across approach from phenazopyridine hydrochloride to Basic Orange 2. Therefore, in addition to the limited mouse carcinogenicity study on Basic Orange 2 (Albert 1956; IARC 1975a, 1987b), the potential carcinogenicity of Basic Orange 2 is also supported by read-across to phenazopyridine hydrochloridide and is considered to also apply to

the related salts of Basic Orange 2 (CAS RNs 63681-54-9 and 75660-25-2, and 79234-33-6[7]) in addition to the free base form, Solvent Orange 3[8].

7.2.3 Health Effects Data for Subsets B (CAS RNs 14408-20-9, 36986-04-6 and 59709-10-3) and C (CAS RNs 68929-07-7 and 69852-41-1), CAS RN 52769-39-8 and CAS RN 93783-70-1

None of these seven substances has been classified for human health effects by any national or international agencies. In addition, none of the postulated azo bond cleavage products from these substances was identified to be classified for human health effects. No studies were identified for carcinogenicity or genotoxicity except for *in vitro* genotoxicity for CAS RN 68929-07-7. Positive results were obtained for this substance in a Prival-modified Ames assay under standard (uninduced S9) and reduced (flavin mononucleotide) conditions in *S. typhimurium* TA98, whereas the results were negative in TA100 (BioReliance 2012).

Among the postulated azo reductive cleavage products of these seven substances, presented in Appendix C, Table C1, six of 12 could be identified for evaluation. Multiple animal studies on the carcinogenicity of 2,6-dichloro-4-nitroaniline (CAS RN 99-30-9) and *N,N*-dimethyl-*p*-phenylenediamine (CAS RN 99-98-9) indicated negative results (FAO/WHO 1998; BG Chemie 1998). *In vivo* genotoxicity data for 2,6-dichloro-4-nitroaniline were identified to be all negative (FAO/WHO 1998), although the results of *in vitro* genotoxicity results for 2,6-dichloro-4-nitroaniline, *N,N*-dimethyl-*p*-phenylenediamine and 2-chloro-4-nitroaniline (CAS 121-87-9) had both positive and negative results in a variety of assays (Appendix C, Table C3).

Results for postulated azo reductive cleavage products were not indicative of a carcinogenic response and were not clearly genotoxic (positive results *in vitro* not seen *in vivo*). Therefore, other methods for characterization were utilized and the postulated azo reductive cleavage products were not considered for risk characterization.

Consideration of analogues

Due to a lack of data available for the seven substances, the OECD QSAR Toolbox (2013) and the C.I. International database (CII 2011) were used to identify structurally similar substances. Subsequent searches of TOXLINE (2013) and SciFinder (2013) were conducted to identify empirical data for read-across purposes. Structural similarity

[7] 79234-33-6 (acetate) is also a related salt of Basic Orange 2, however no sources of exposure to the general population of Canada were identified for this substance, so it was not specifically considered in the Health Effects section.

[8] Solvent Orange 3 health effects and carcinogenic potential was also characterized in another screening assessment of Azo Solvent Dyes (Environment Canada, Health Canada 2015a)

as well as physical and chemical properties was considered for those substances with data identified for read-across through these search strategies.

The OECD QSAR Toolbox (2013) was searched for the following functional groups: aromatic amine, aryl, azo; aniline, aminoaniline, ortho (subset A); nitrobenzene, pyridine, aryl halide (subset B); benzothiazole/benzoisothiazole, ether, alcohol (subset C); amidine, isopropyl, thiadiazole (CAS RN 93783-70-1); benzyl, triazole (CAS RN 52769-39-8). This search identified 44 substances, of which 3 had data identified through subsequent searches of TOXLINE (2013) and SciFinder (2013). Of these three substances, two were determined to have adequate data, CAS RN 83969-12-4 (methyl sulfate salt) and CAS RN 66693-25-2 (sulfate salt). CAS RN 83969-12-4 was similar to CAS RN 93783-70-1, whereas CAS RN 66693-25-2 was similar to CAS RN 68929-07-7 (subset C), as determined by Tanimoto coefficient values meeting 100% (OECD QSAR Toolbox 2013). CAS RN 66693-25-2 was a dimer, resulting in a higher molar weight, and an isomer of CAS RN 68929-07-7, where the location of the methoxy substituent on the benzothiazolium ring is different. CAS RN 83969-12-4 is a methyl sulfate salt, whereas CAS RN 93783-70-1 is a zinc chloride salt. This difference would affect the water solubility of these substances. Based on physical and chemical properties such as molar weight and water solubility, the substances identified from this OECD QSAR Toolbox (2013) search were not considered to be appropriate for use as similar substances for read-across purposes.

The C.I. International database (CII 2011) was also searched for azo basic dyes, followed by a manual verification of the chemical structure for an aromatic azo bond or its structural tautomer (hydrazone). This search identified 31 substances, out of which 11 were identified as having some toxicity data through subsequent searches in TOXLINE (2013) and SciFinder (2013). Among these 11 substances, only 2 substances, Basic Brown 17 (CAS RN 68391-32-2) and Basic Brown 16 (CAS RN 26381-41-9), were identified to have relevant data. However, these substances were not considered structurally similar enough to any of the seven substances, with Tanimoto coefficient values ranging only from 12% to 32% (OECD QSAR Toolbox 2013). Visual verification also indicated notable differences in core chemical structure as well as functional groups.

Overall, none of the substances identified above was considered appropriate to inform either subset B or C or any of the seven substances individually through read-across.

Limited empirical data were available for the substances in subset B or C or CAS RN 93783-70-1 and CAS RN 52769-39-8. A search was conducted for analogues using the OECD QSAR Toolbox (2013), the C.I. International database (CII 2011), TOXLINE (2013) and SciFinder (2013) for the purposes of read-across, where structural similarities and physical and chemical properties were considered; however, no appropriate analogues were identified. There were no indications of effects of concern, such as classifications of carcinogenicity or genotoxicity by national or international agencies, for the azo reductive cleavage products of these six substances. Since the focus of the Azo and Benzidine-based Substance Grouping was generally on

genotoxicity and carcinogenicity, this does not consider the potential for effects being reported for other endpoints based on exposure to the possible azo reductive cleavage products of these seven substances. Because of limited empirical and read-across information available for subset B, subset C, CAS RN 52769-39-8 and CAS RN 93783-70-1, it is conservatively considered that the overall hazard for those substances is similar to that of subset A.

7.2.4 Health Effects Data for the Twenty remaining Azo Basic Dyes

No previous reviews or assessments conducted by other national or international jurisdictions were identified for the remaining 20 Azo Basic Dyes. Among the postulated azo bond cleavage products of these 20 substances, one EU22 amine, *p*-aminoazobenzene (CAS RN 60-09-3), was identified as the postulated azo bond cleavage product of CAS RN 75199-20-1 and CAS RN 125329-01-3. CAS RNs 63681-54-9, 75199-20-1, 79234-33-6 and 125329-01-3 were identified to release aniline (CAS RN 62-53-3) as the postulated azo bond cleavage product, which has previously been evaluated by the Government of Canada (Health Canada 2011a). Effects of aniline are summarized in Section 7.2.2 and Appendix C Table C2.

7.2.5 Uncertainties in health effects assessment

Overall, data availability was limited. Read-across from substances with available data was applied for subset A, while there was a paucity of information for subset B, subset C, CAS RN 93783-70-1 and CAS RN 52769-39-8 compared with subset A. Consideration of analogues for subset B, subset C, CAS RN 93783-70-1 and CAS RN 52769-39-8 was not appropriate because of lack of structural similarity. This lack of data contributes to significantly higher uncertainty and lower confidence for these health effects assessments relative to the subset A assessment. Among the identified studies, limitations such as unreported purity and identity (supplier) of the test substance, and the age of the study, as well as the methodology were also noted. In addition, there are concerns regarding the quality and reproducibility of the Albert (1956) study (Searle and Teale, 1984, BAuA, 2003). in a letter to the Lancet, Searle and Teale (1984) indicate that Albert was not able to replicate his previous results (1956) when using fresh Basic Orange 2 (factory or chemist-prepared sources), however, no additional information was available to further evaluate this study.

Limited information is available to inform azo bond cleavage potential of the Azo Basic Dyes. The reductive cleavage of the Azo Basic Dyes is based primarily on results from two substances, for read-across to a number of structurally diverse substances. As there is no similar functional group or structural similarity across the Azo Basic Dyes, there is low confidence in the resulting conclusion for all Azo Basic Dyes.

Although limited in available studies, differences between the types of neoplasms and non-neoplastic effects were observed in the same species, as well as a lack of replication between species when experimental animals are treated with phenazopyridine hydrochloride. These differences may be due either to the differences

between species or the treatment. One study was identified that compares the metabolism of phenazopyridine hydrochloride between species, including humans, rats, mice and hamsters (Thomas et al. 1990). Even on a metabolic level, there are differences to be considered. With regard to the non-cancer effects of subset A, the Cambel et al. (1954) short-term drinking water study of Basic Orange 2 examined only blood and stomach after a single-dose level, whereas the NCI (1978) chronic oral (feed) multiple dose study design did not include clinical chemistry tests, including hematological parameters (NCI 1978). The absence of comparable data in these studies, in addition to the different duration of exposure and carrier (water vs. food) presents challenges in the evaluation of available information.

7.3 Characterization of Risk to Human Health

For 20 of the 32 Azo Basic Dyes included in the human health assessment, available information did not identify sources of current exposure for the general population of Canada, therefore risk to human health is not expected for these substances. Therefore, the risk characterization focusses on the other 12 Azo Basic Dyes which were identified as being in commerce in Canada.

Exposure to 12 of the 32 Azo Basic Dyes from environmental media is not expected to be a significant source of exposure to the general population of Canada based on physical and chemical properties and import quantities for these substances. Among these 12 substances, exposure to the general population from phenazopyridine hydrochloride in environmental media based on releases from the therapeutic use in a pharmaceutical formulation is expected to be negligible. Therefore risk to human health is considered low from environmental media for these 12 substances.

The predominant source of exposure to these 12 Azo Basic dyes is through cosmetic and consumer product use, specifically hair dye, paper, pen ink and textiles. Risk to human health from exposure via these uses has been characterized for these 12 substances in the following sections.

Subset A (Phenazopyridine hydrochloride, Basic Orange 2 and related salts (CAS RNs 63681-54-9 and 75660-25-2), Basic Brown 1)

Three of the five substances in Subset A (Basic Brown 1, Basic Orange 2 and CAS RN 75660-25-2) were considered to have potential exposure through use of consumer products based on available data, and risk to human health from these exposures has been characterized below.

Carcinogenicity is considered to be the critical health effect for some of the substances in Subset A, based on read-across of empirical data for phenazopyridine hydrochloride. However, no uses of the three Subset A substances (Basic Brown 1, Basic Orange 2 and CAS RN 75660-25-2) were identified which would result in chronic exposure scenarios, therefore no margins of exposure for carcingocencity were derived for these substances. Since exposure to the general population of Canada for these three

substances is expected from use of products that are used intermittently rather than frequently or daily, the risk characterization for Subset A is based on comparison with non-cancer effects.

Exposure to Basic Orange 2 in hair dye for the general population of Canada via the dermal route was estimated to range from 0.15 to 1.4 mg/kg-bw per event. As neither health effect study was conducted by the dermal route of administration, the effects documented in the oral single dose (160 mg/kg-bw per day) short-term Basic Orange 2 study was considered to be an appropriate and protective point of departure for risk characterization for exposures via the dermal route. This is based on the conservative assumption that dermal and oral absorption are equivalent. Comparison of estimates for dermal exposure to Basic Orange 2 via hair dye with the oral short-term adverse effect level in rats results in margins of exposure (MOEs) ranging from 114 to 1066. These MOEs are considered adequate to address uncertainties in the exposure and health effects databases.

Incidental combined oral and dermal exposure to toddlers for the general population of Canada to Basic Orange 2 in ballpoint pen ink was estimated as 0.002 mg/kg bw per event (Appendix B). Comparison of estimates for oral exposure to Basic Orange 2 via ballpoint pen ink with the oral short-term effect level in rats results in a MOE of 80 000. This MOE is considered adequate to address uncertainties in the exposure and health effects databases.

For Basic Brown 1 and CASR RN 75660-25-2, exposure through use in paper products is expected. For CAS RN 75660-25-2, oral mouthing by toddlers is considered to be a conservative exposure scenario for paper use, however due to uncertainty regarding in the actual amount ingested, the dye fastness to the paper following oral ingestion, and the incidental nature of the scenario, exposure is considered to be low. Basic Brown 1 was also reported as being in paper products, but it is not expected in products available to children, therefore oral exposure to toddlers from this substance is not expected. Overall, use of Basic Brown 1 and CAS RN 75660-25-2 in paper is not expected to result in a significant source of exposure to the general population and therefore the risk to human health is considered low for this use.

For two of the five remaining substances in Subset A (phenazopyridine hydrochloride and CAS RN 63681-54-9), exposures to the general population are not expected. For phenazopyridine hydrochloride, uses of this substance previously assessed under the *Food and Drugs Act* are not considered in this assessment, and no other general population exposures to this substance have been identified. For CAS-RN 63681-54-9, the use reported under section 71 was found to know longer be present in the Canadian market. As available information did not identify sources of current exposure to these substances for the general population of Canada, risk to human health is not expected for these substances.

Subsets B (CAS RNs 14408-20-9, 36986-04-6 and 59709-10-3) and C (CAS RN 68929-07-7 and CAS RN 69852-41-1), CAS RN 52769-39-8 and CAS RN 93783-70-1

Exposure of the general population of Canada to Azo Basic Dyes in subsets B, C, CAS RN 52769-39-8 and CAS RN 93783-70-1 is expected to be through incidental oral ingestion of paper by toddlers and dermal contact with textiles by adults and infants as well as oral mouthing of textiles by infants. There is also limited of empirical and read-across information for subsets B, C, CAS RN 52769-39-8 and CAS RN 93783-70-1. Although there is too great uncertainty to formally apply a read-across from Subset A to subsets B, C, CAS RN 52769-39-8 and CAS RN 93783-70-1, in the absence of data for these substances, health effect levels from Subset A have been applied as a conservative approach for deriving risk estimates.

For the use of these substances in paper, it is considered that the risk to human health for subsets B, C, CAS RN 52769-39-8 and CAS RN 93783-70-1 would be no more than that of subset A. As such, the use of these substances in paper is not expected to result in a significant source of exposure to the general population and therefore the risk to human health is considered low for this use.

For evaluation of dermal exposure to adults and infants to textiles from Azo Basic Dyes in subsets B, C, CAS RN 52769-39-8 and CAS RN 93783-70-1, a comparison of the critical effect levels from Subset A (i.e. phenazopyridine hydrochloride) have been applied. Based on the lowest dose for tumour formation for phenazopyridine hydrochloride (150 mg/kg-bw/day for liver tumour formation in mice exposed to phenazopyridine hydrochloride) and chronic dermal exposure from textiles (2.6-4.0 µg/kg bw/day) (Environment Canada and Health Canada 2014a) represents a theoretical conservative scenario. The resulting MOEs (37500 – 57700) are adequate to address uncertainties in the exposure and health effects databases. Similarly, as the estimate for oral mouthing from infants results in exposures lower than that for the dermal scenario presented above ($2.7x10^{-2}$ µg/kg bw/day) (Environment Canada and Health Canada 2014a) the MOEs for this scenario are also considered to be adequate.

Uncertainties

Overall, health effects data availability is limited for Azo Basic Dyes. There were also limited data relating to physical and chemical properties and azo reductive cleavage.

Read-across was applied in Subset A from data-rich substances to those with more limited toxicity data, based on structural similarities and physical and chemical properties.

Combination of the limited data availability for subsets B and C, CAS RN 93783-70-1 and CAS RN 52769-39-8 and lack of suitably similar substances with data for read-across resulted in high uncertainty with regard to the hazard potential of these substances and consequently, the lower confidence in those datasets relative to Subset A. Additionally, exposure estimates for these substances are based on limited

information indicating that they are used in paper and textile products. However, there is reasonable confidence that using the health effects levels from Subset A represent a conservative approach to estimate the risk for these substances.

With regard to paper ingestion by toddlers, there is uncertainty regarding the assumptions in the scenario, such as quantity of paper ingested by a toddler during a mouthing event and bioavailability of these substances from paper. However, it is considered that due to uncertainty regarding in the actual amount ingested, the dye fastness to the paper following oral ingestion, and the incidental nature of the scenario, exposure is considered to be low for this scenario.

Among the 12 Azo Basic Dyes for which exposure of the Canadian general population is expected, dermal exposure to Basic Orange 2 may occur through use of hair dye. As no dermal toxicity studies were identified, route-to-route extrapolation was used to derive MOEs. An additional uncertainty, probably conservative in nature, is associated with the use of data from short-term studies to derive the MOEs for per event exposure scenarios.

In addition, there is uncertainty due to impurities, residues and variable compositions of these dyes when reported as test material in toxicity studies.

Azo Basic Dyes with Effects of Concern

Overall, human health risk from the substances in this assessment is low based on the current levels of exposure. However as indicated above, some of the Azo Basic Dyes in this assessment have effects of concern based on potential carcinogenicity. A list of these substances is shown in Appendix D.

In the draft version of the Certain Azo Basic Dyes assessment, seven Basic Dyes were identified to have human health effects of concern. Since the draft publication, more precise considerations based principally on lines of evidence for carcinogenic potential were applied to indicate substances which were considered to have effects of concern (Appendix D). As such, one of the seven Basic Dyes previously identified in the draft assessment as having health effects of concern, Basic Brown 1, is no longer considered to meet the lines of evidence for potential carcinogenicity due to a lack of chronic animal studies and uncertainty regarding read-across. In addition, CAS RN 79234-33-6 (related acetate salt of Basic Orange 2) is now also identified to have human health effects of concern based the same lines of evidence as Basic Orange 2 (see Appendix D). This substance was not previously identified for effects of concern in the draft assessment.

8. Conclusion

Considering all available lines of evidence presented in this Screening Assessment, there is low risk of harm to organisms and the broader integrity of the environment from the 33 Azo Basic Dyes evaluated in this assessment. It is concluded that the 33 Azo Basic Dyes do not meet the criteria under paragraphs 64(*a*) or 64(*b*) of CEPA 1999, as they are not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

Based on the available information presented in this Screening Assessment, it is concluded that 32 Azo Basic Dyes evaluated in the human health assessment do not meet the criteria under paragraph 64(*c*) of CEPA 1999 as they are not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health.

In addition, there are no updates to the assessment and conclusion made with respect to paragraph 64(*c*) for NDTHPM, previously considered by the Government of Canada under the Challenge Initiative of the CMP.

It is concluded that 33 Azo Basic Dyes evaluated in this assessment do not meet any of the criteria set out in section 64 of CEPA 1999.

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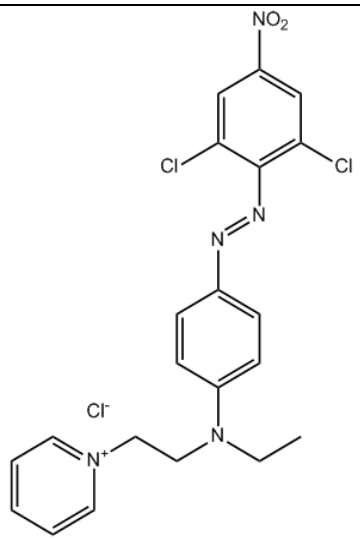
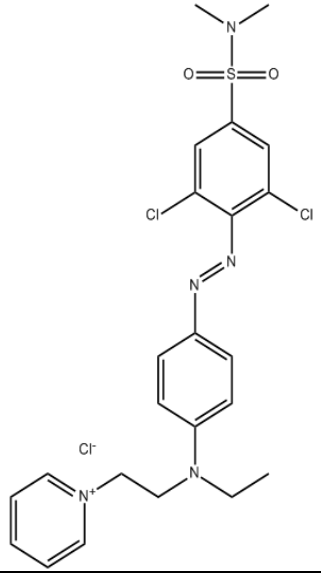
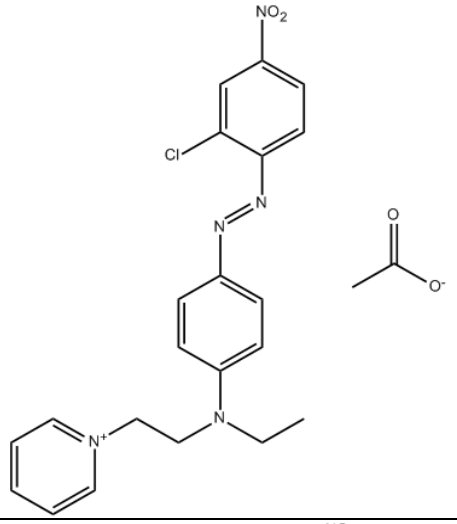
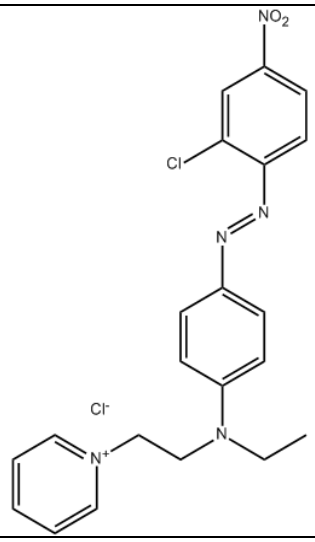
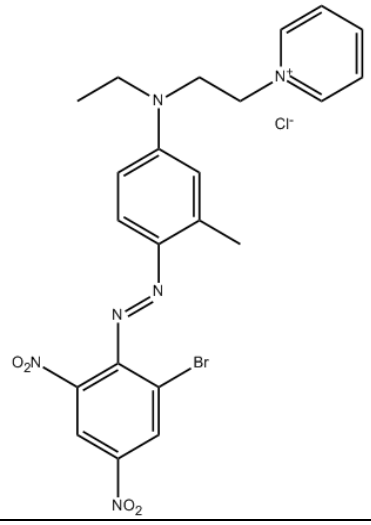
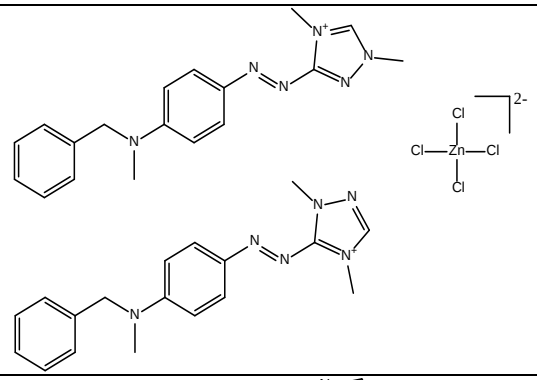
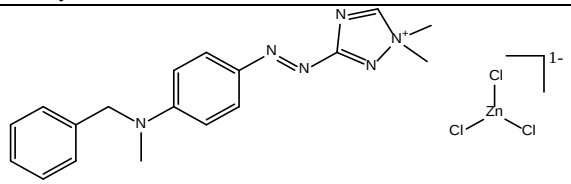
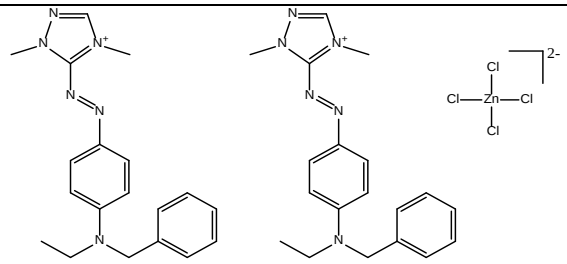
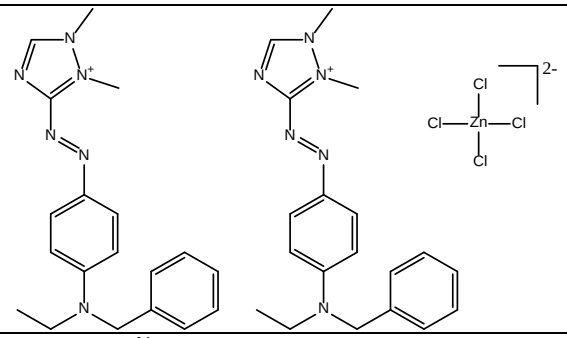
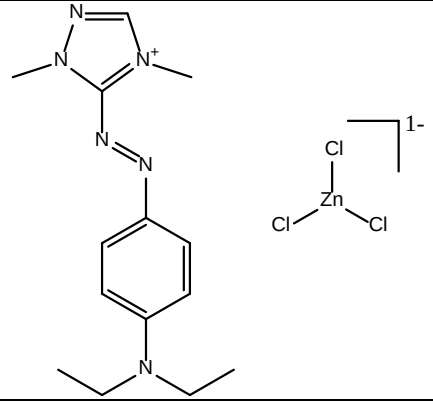
Appendices

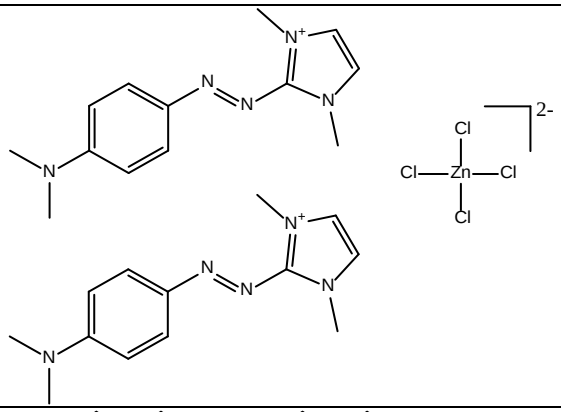
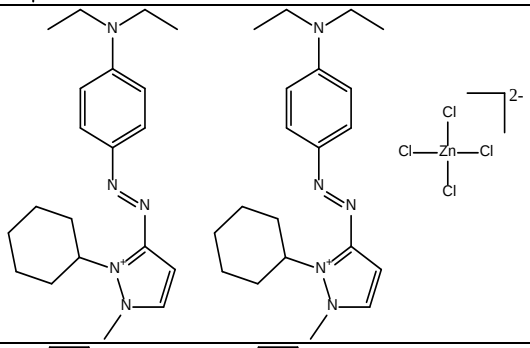
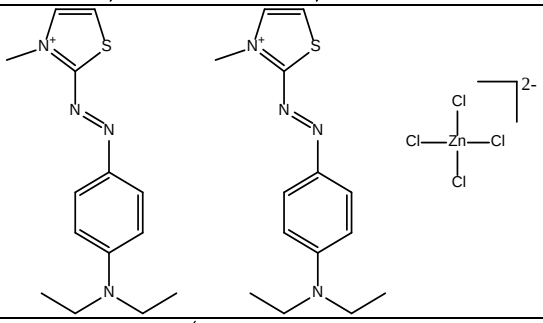
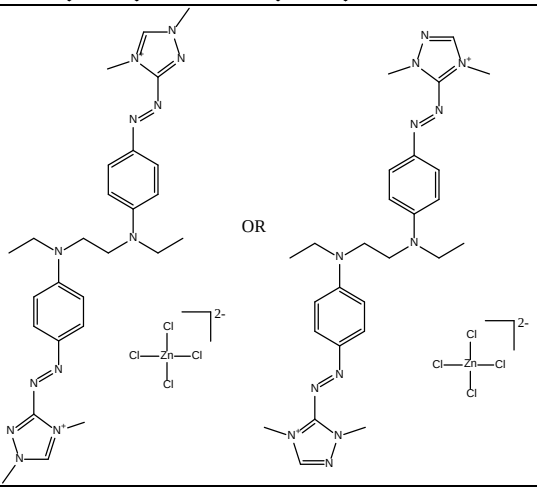
Appendix A: Supplementary Data Tables

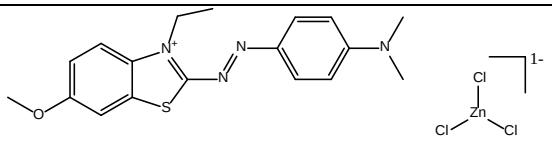
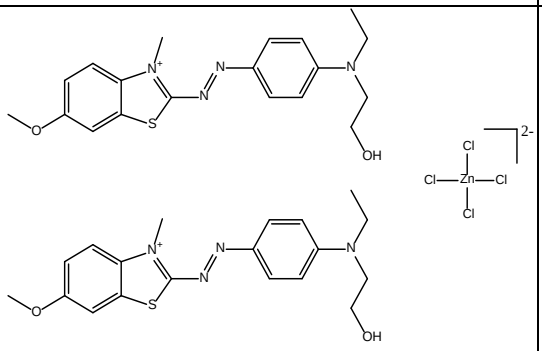
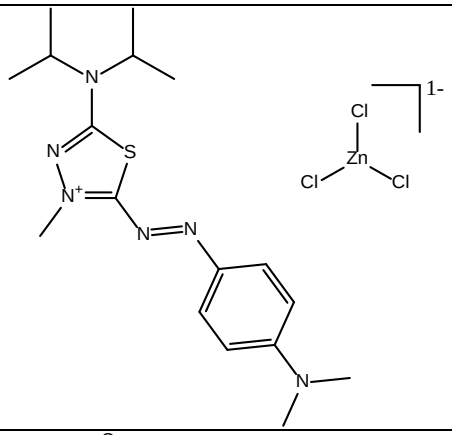
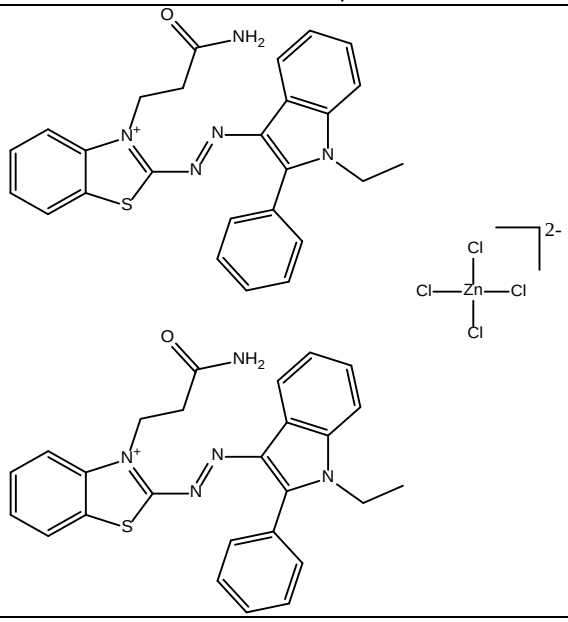
Table A1.Substance identities for individual Azo Basic Dyes

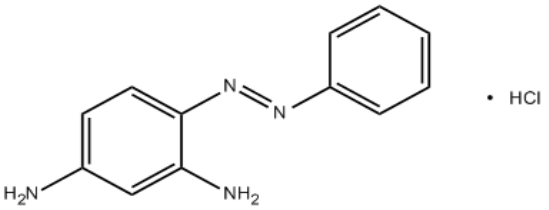
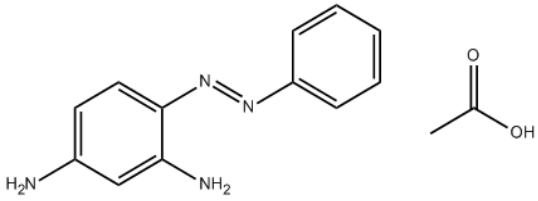
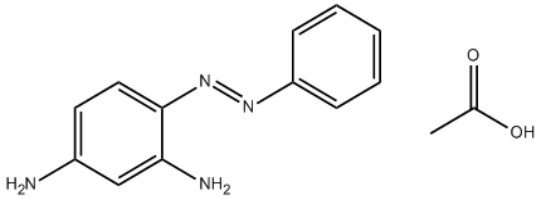
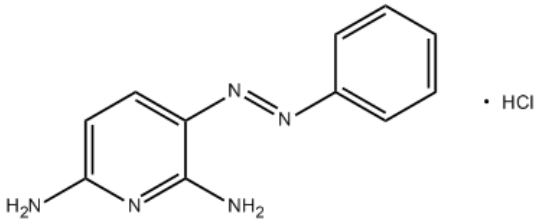
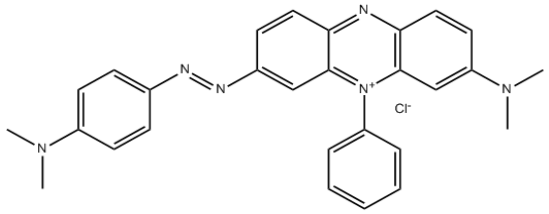
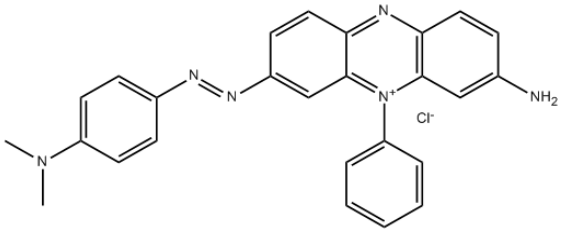
Eco-subset	CAS RN / C.I. name	Chemical structure	Chemical formula (molar weight, g/mol)
1	14408-20-9		$C_{21}H_{20}Cl_2N_5O_2 \cdot Cl$ (481)
1	10189-42-1		$C_{23}H_{26}Cl_2N_5O_2S \cdot Cl$ (543)

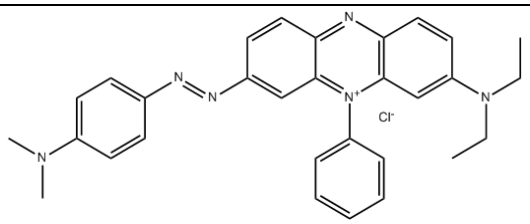
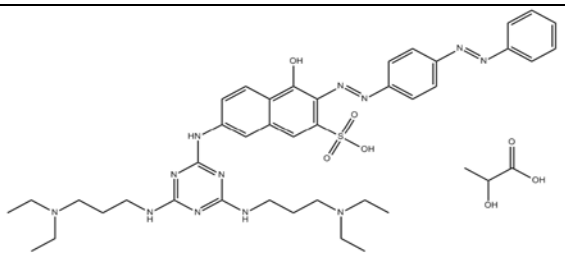
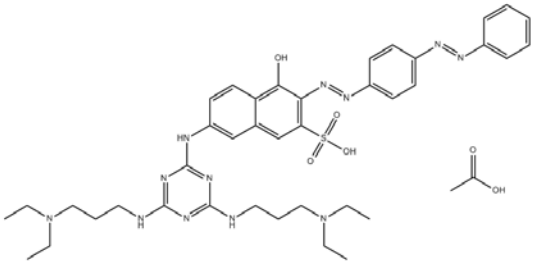
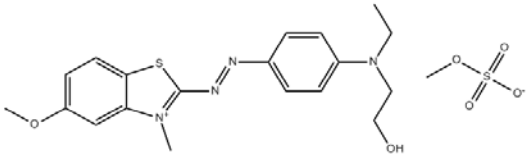
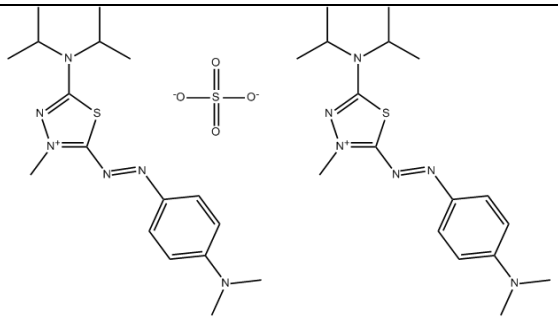
1	59709-10-3		$C_{21}H_{21}ClN_5O_2 \cdot C_2H_3O_2$ (470)
1	36986-04-6		$C_{21}H_{21}ClN_5O_2 \cdot Cl$ (446)
1	72361-40-1		$C_{22}H_{22}BrN_6O_4 \cdot Cl$ (550)

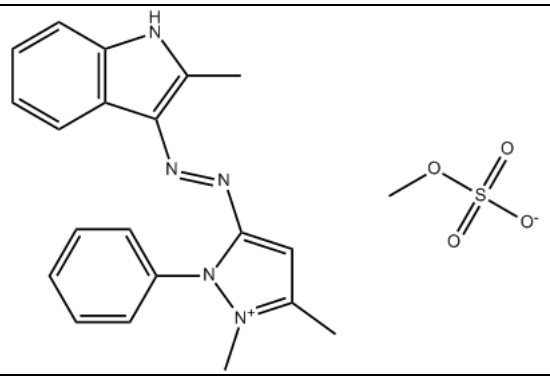
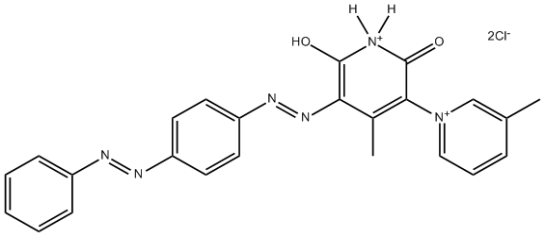
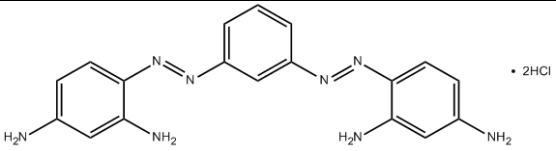
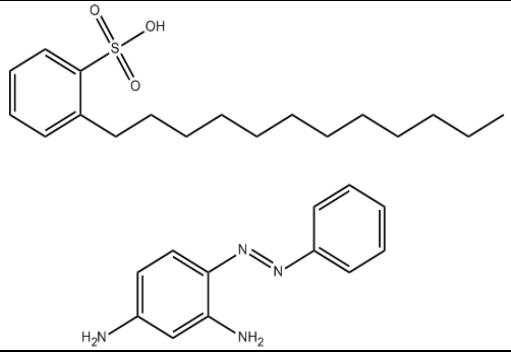
2	85114-37-0		$2C_{18}H_{21}N_6 \cdot Cl_4Zn$ (854)
2	52769-39-8		$C_{18}H_{21}N_6 \cdot Cl_3Zn$ (493)
2	72379-36-3		$2C_{19}H_{23}N_6 \cdot Cl_4Zn$ (878)
2	72379-37-4		$2C_{19}H_{23}N_6 \cdot Cl_4Zn$ (878)
2	14970-39-9		$C_{14}H_{21}N_6 \cdot Cl_3Zn$ (447)

2	68936-17-4		$2C_{13}H_{18}N_5 \cdot Cl_4Zn$ (696)
2	63589-49-1		$2C_{20}H_{30}N_5 \cdot Cl_4Zn$ (888)
2	65150-98-3		$2C_{14}H_{19}N_4S \cdot Cl_4Zn$ (760)
2	74744-63-1		$C_{26}H_{36}N_{12} \cdot Cl_4Zn$ (724)

2	23408-72-2		$C_{18}H_{21}N_4OS \cdot Cl_3Zn$ (513)
2	69852-41-1		$2C_{19}H_{23}N_4O_2S \cdot Cl_4Zn$ (950)
2	93783-70-1		$C_{17}H_{27}N_6S \cdot Cl_3Zn$ (519)
2	85480-88-2		$C_{52}H_{48}N_{10}O_2S_2 \cdot Cl_4Zn$ (1116.33)

3	532-82-1 Basic Orange 2		$C_{12}H_{12}N_4 \cdot ClH$ (249)
3	79234-33-6		$C_{12}H_{12}N_4 \cdot C_2H_4O_2$ (272)
3	75660-25-2		$C_{12}H_{12}N_4 \cdot C_2H_4O_2$ (272)
3	phenazopyridine hydrochloride		$C_{11}H_{12}ClN_5$ (250)
4	4608-12-2		$C_{28}H_{27}N_6 \cdot Cl$ (483)
4	4618-88-6		$C_{26}H_{23}N_6 \cdot Cl$ (455)

4	2869-83-2		$C_{30}H_{31}N_6 \cdot Cl$ (511)
5	125329-01-3		$C_{39}H_{50}N_{12}O_4S \cdot C_3H_6O_3$ (873)
5	71032-95-6 NDTHPM		$C_{39}H_{50}N_{12}O_4S \cdot C_2H_4O_2$ (843)
6	68929-07-7		$C_{19}H_{23}N_4O_2S \cdot CH_3O_4S$ (483)
6	83969-13-5		$2C_{17}H_{27}N_6S \cdot O_4S$ (791)

6	29508-48-3		$C_{20}H_{20}N_5 \cdot CH_3O_4S$ (444)
7	75199-20-1		$C_{24}H_{21}N_6O_2 \cdot Cl$ (462)
7	10114-58-6 Basic Brown 1		$C_{18}H_{18}N_8 \cdot 2ClH$ (419)
7	63681-54-9		$C_{18}H_{30}O_3S \cdot C_{12}H_{12}N_4$ (539)

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index

Table A2: Experimental physical and chemical properties for eco-subset 1 (with data) including substances used for read-across

CAS RN	Property	Value	Reference
6257-64-3 (Read-across for melting point, boiling point, vapour pressure and log K_{ow})	Melting point	276–278°C	Sigma-Aldrich 2009
6257-64-3 (Read-across for melting point, boiling point, vapour pressure and log K_{ow})	Boiling point	> 100°C	Institute of Paper Science Technology Inc. 2009

CAS RN	Property	Value	Reference
6257-64-3 (Read-across for melting point, boiling point, vapour pressure and log K_{ow})	Vapour pressure	~18 mmHg[a] at 20°C	Institute of Paper Science Technology Inc. 2009
6257-64-3 (Read-across for melting point, boiling point, vapour pressure and log K_{ow})	Log K_{ow}	2.03	Tonogai et al. 1982

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index; K_{ow}, octanol–water partition coefficient

[a] 1 mmHg = 133.3 Pa.

Table A3: Experimental physical and chemical properties for eco-subset 2 (with data) including substances used for read-across

CAS RN	Property	Value	Reference
64071-86-9 (Read-across for melting point and solubility)	Solubility in water	10 000 mg/L	Santa Cruz Biotechnology Inc. 2013a
64071-86-9 (Read-across for melting point and solubility)	Solubility in water	60 000 mg/L	Green 1990
64071-86-9 (Read-across for melting point and solubility)	Solubility in ethanol	3000 mg/L	Green 1990
64071-86-9 (Read-across for melting point and solubility)	Melting point	150°C	Green 1990
64071-86-9 (Read-across for melting point and solubility)	Melting point	149–151°C	Avocado Research Chemicals Ltd. 1998
61966-14-1 (Read-across for melting point and solubility)	Solubility in water	20 000 mg/L	Green 1990
61966-14-1 (Read-across for melting point and solubility)	Solubility in ethanol	5000 mg/L	Green 1990
61966-14-1 (Read-across for melting point and solubility)	Melting point	147°C	Green 1990

Abbreviation: CAS RN, Chemical Abstracts Service Registry Number

Table A4: Experimental physical and chemical properties for eco-subset 3 (with data) including substances used for read-across

CAS RN / C.I. name	Property	Value	Reference
Basic Orange 2	Melting point	118.00–118.50°C	Budavari 1996
Basic Orange 2	Melting point	118.00–118.50°C	Sabnis 2008
Basic Orange 2	Melting point	235°C	Green 1990
Basic Orange 2	Boiling point	2262°C	Lide 1995–1996
Basic Orange 2	Boiling point	2262°C	Sabnis 2008
Basic Orange 2	Solubility in water	20 000 mg/L	Green 1990
Basic Orange 2	Solubility in water	5.5% at 15°C	Budavari 1996
Basic Orange 2	Solubility in absolute ethanol	47 500 mg/L	Budavari 1996
Basic Orange 2	Solubility in ethanol	9000 mg/L	Green 1990
phenazopyridine hydrochloride	Melting point	244–245°C	NCI 1978
136-40-3	Melting point	235°C	IARC 1975b
136-40-3	Solubility in water	100–1000 mg/L at 20°C	NTP 1992b
136-40-3	Solubility in water	15.9 g/L at 25°C	ChemIDplus 1993–
136-40-3	Solubility in cold water	3 mg/L	Budavari 1996
136-40-3	Solubility in boiling water	50 mg/L	Budavari 1996
136-40-3	Log K_{ow}	–0.30	ChemIDplus 1993–
136-40-3	Vapour pressure	3.51×10^{-11} mmHg[a] at 25°C	ChemIDplus 1993–
136-40-3	Vapour pressure	4.57×10^{-12} mmHg[a] at 25°C	New Jersey Department of Health and Senior Services 2001

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index; K_{ow}, octanol–water partition coefficient

[a] 1 mmHg = 133.3 Pa.

Table A5: Experimental physical and chemical properties for eco-subset 4 (with data) including substances used for read-across

CAS RN	Property	Value	Reference
2869-83-2	Solubility in water	30 000 mg/L	Green 1990
2869-83-2	Solubility in ethanol	5000 mg/L	Green 1990
2869-83-2	Melting point	240°C	Alfa Aesar 2007
2869-83-2	Melting point	> 200°C	Sabnis 2010

Abbreviation: CAS RN, Chemical Abstracts Service Registry Number

Table A6: Experimental physical and chemical properties for eco-subset 5 (with data) including substances used for read-across

CAS RN / C.I. name	Property	Value	Reference
71032-95-6 NDTHPM	Solubility in alcohol and acetone	Soluble	Study Submission 2006
118658-98-3 Basic Red 111 (Read-across for log K_{ow}, solubility and biodegradation)	Solubility in *n*-octanol	160 000 mg/L	Study Submission 2009e
118658-98-3 Basic Red 111 (Read-across for log K_{ow}, solubility and biodegradation)	Solubility in water	> 340 000 mg/L	Study Submission 2009e
118658-98-3 Basic Red 111 (Read-across for log K_{ow}, solubility and biodegradation)	Log K_{ow}	< −0.33	Study Submission 2009e

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index; K_{ow}, octanol–water partition coefficient

Table A7: Experimental physical and chemical properties for eco-subset 6 (with data) including substances used for read-across

CAS RN / C.I. name	Property	Value	Reference
12270-13-2 Basic Blue 41 (Read-across for decomposition point, melting point and biodegradation)	Decomposition point	> 250°C	M. Dohmen USA Inc. 2009
12270-13-2 Basic Blue 41 (Read-across for decomposition point, melting point and biodegradation)	Melting point	> 120°C	M. Dohmen USA Inc. 2009
15000-59-6 Basic Blue 54 (Read-across for solubility)	Solubility in water	140 mg/L	Baughman et al. 1996

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index

Table A8: Experimental physical and chemical properties for eco-subset 7 (with data) including substances used for read-across

CAS RN / C.I. name	Property	Value	Reference
Basic Brown 1	Log K_{ow}	2	Tonogai et al. 1982
Basic Brown 1	Solubility in water	1000–10 000 mg/L at 20°C	NTP 1992b
Basic Brown 1	Solubility in water	50 000 mg/L	Green 1990
Basic Brown 1	Solubility in water	10 000 mg/L	Santa Cruz Biotechnology Inc. 2013b
Basic Brown 1	Solubility in ethanol	5000 mg/L	Green 1990
Basic Brown 1	pK_a	5	Sabnis 2010
Basic Brown 1	Melting point	> 200°C	Sabnis 2010
Basic Brown 1	Melting point	220°C	NTP 1992b
Basic Brown 1	Melting point	220°C (decomposes)	Columbus Chemical Industries, Inc. 2012
Basic Brown 1	Melting point	> 280°C	MP Biomedicals. 2006
Basic Brown 1	Vapour pressure	≤ 0.0001 kPa at 25°C	Mallinckrodt Baker Inc. 2010
63681-54-9	Decomposition point	> 350 °C	BASF Canada 2004
63681-54-9	Solubility in water	< 1000 mg/L at 20°C	BASF Canada 2004

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index; K_{ow}, octanol–water partition coefficient; pK_a, acid dissociation constant

Table A9: Summary of modelled data for degradation of Azo Basic Dyes[a]

Fate process (compartment)	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Atmospheric oxidation (air)	AOPWIN 2010[b]	$t_{½}$ = 0.025–1.081 days (outlier CAS RN phenazopyridine hydrochloride, $t_{½}$ = 4.273 days)	< 2
Ozone reaction (air)	AOPWIN 2010[b]	N/A[c] (except CAS RN 75199-20-1, $t_{½}$ = 0.02 day)	N/A
Hydrolysis (water)	HYDROWIN 2010[b]	Not in training set (except CAS RN 75199-20-1; see persistence section)	N/A
Primary biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2010[b] Submodel 4: Expert survey (qualitative results)	1.94–3.35[d] (biodegrades slowly; borderline *n* = 9)	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2010[b] Submodel 3: Expert survey (qualitative results)	0.08–2.24[d] (biodegrades very slowly; borderline *n* = 4)	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2010[b] Submodel 5: MITI linear probability	−0.99 to −0.17[e] (biodegrades very slowly)	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2010[b] Submodel 6: MITI non-linear probability	0[e] (biodegrades very slowly)	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	DS TOPKAT ©2005–2009 Probability	N/A	?
Ultimate biodegradation: Biodegradation (aerobic) (water)	CATALOGIC 2012 % BOD	% BOD = 0.29–19.78[f] (biodegrades slowly)	≥ 182

Abbreviations: BOD, biological oxygen demand; CAS RN, Chemical Abstracts Service Registry Number; MITI, Ministry of International Trade & Industry (Japan); N/A, not applicable; $t_{½}$, half-life

[a] Substances used in this summary include the following CAS RNs: Basic Orange 2, 4608-12-2, 10189-42-1, 14408-20-9, phenazopyridine hydrochloride, 2869-83-2, 4618-88-6, Basic Brown 1, 29508-48-3, 36986-04-6, 59709-10-3, 63681-54-9, 68929-07-7, NDTHPM, 72361-40-1, 75199-20-1, 75660-25-2, 79234-33-6, 83969-13-5 and 125329-01-3.

[b] EPI Suite (2012).

[c] Model does not provide an estimate for this type of structure.

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

[e] Output is a probability score.

[f] Substances used in this range also include CAS RNs 85114-37-0, 93783-70-1, 14970-39-9, 52769-39-8, 63589-49-1, 69852-41-1, 72379-37-4 and 23408-72-2.

Appendix B: Estimated Exposures from Use of Products

Exposures from use of products were estimated for different age groups based on body weights from Health Canada's report on exposure factors for assessing total daily intake of priority substances by the general population of Canada (Health Canada 1998):

- Infant (0–6 months): 7.5 kg
- Toddler (0.5–4 years): 15.5 kg
- Child (5–11 years): 21.0 kg
- Teenager (12–19 years): 59.4 kg
- Adult (20–59 years): 70.9 kg
- Senior (60+ years): 72.0 kg

Table B1: Dermal exposure to Basic Orange 2 from use in cosmetic products

Cosmetic product scenario	Concentration (%)	Daily exposure (mg/kg-bw per day)	Per event exposure (mg/kg-bw)
Hair dye (semi-permanent)	0.3–1	0.02–0.07	0.15–0.49
Hair dye (permanent)	0.3–1	0.009–0.031	0.42–1.4

Dermal parameters

Product scenario	Assumptions[a]
Hair dye – non-spray/wash-in; permanent	Exposure frequency: 0.02/day (7.99/year) (Statistics Canada, 2012) Product amount: 100 g/application Overall retention factor: 0.10 (SCCS 2011)
Hair dye – non-spray/wash-in; semi-permanent	Exposure frequency: 0.14 (1/week) (SCCS 2011) Product amount: 35 g/application (SCCS 2011) Overall retention factor: 0.1 (professional judgement)

[a]All assumptions were ConsExpo default assumptions (RIVM 2006a) unless otherwise noted.

Incidental dermal and oral exposure estimates to Basic Orange 2 from ballpoint pen ink

This scenario covers both dermal and incidental oral exposure from hand-to-mouth activity by a toddler. The Art and Creative Materials Institute, Duke University, Durham, North Carolina, reported the assumption that a child will absorb 25 cm of ink line daily either through dermal or incidental oral exposure (2009 personal communication from Art and Creative Materials Institute to Existing Substances Risk Assessment Bureau, Health Canada; unreferenced). A default ink laydown rate of 100 µg/cm (90th percentile level ink laydown of writing instruments; 2009 personal communication from Art and Creative Materials Institute to Existing Substances Risk Assessment Bureau, Health Canada; unreferenced) was used. A default dermal absorption fraction of 100% was assumed. Overall, the resulting exposure estimate is expected to be conservative.

Estimated Per Event Exposure via Dermal Route

$$= \frac{\textit{Daily ink line} \times \textit{Ink laydown rate} \times \textit{Conc} \times \textit{Dermal Abs}}{\textit{BW}}$$

Daily ink line:	25 cm
Ink laydown rate:	100 µg/cm
Concentration (*Conc*):	1.5% w/w (Smoothline Writing Instruments, 2010)
Dermal absorption fraction (*Dermal Abs*):	100% (default)
Body weight (*BW*):	15.5 kg (toddler)

Estimated Per Event Dermal Exposure = 2.4×10^{-3} mg/kg-bw

Estimated Per Event Oral Exposure = 2.4×10^{-3} mg/kg-bw

Appendix C: Supplementary Health Information

Table C1: Postulated azo bond cleavage products for subsets A, B and C and CAS RNs 93783-70-1 and 52769-39-8

Subset	Substance (C.I. name or CAS RN)	Postulated azo bond cleavage product (name)	Postulated azo bond cleavage product (CAS RN)
A	Phenazopyridine hydrochloride	Aniline	62-53-3
A	Phenazopyridine hydrochloride	2,3,6-Triaminopyridine	4318-79-0
A	Basic Orange 2	Aniline	62-53-3
A	Basic Orange 2	1,2,4-Triaminobenzene	615-71-4
A	63681-54-9	Aniline	62-53-3
A	63681-54-9	1,2,4-Triaminobenzene	615-71-4
A	75660-25-2	Aniline	62-53-3
A	75660-25-2	1,2,4-Triaminobenzene	615-71-4
A	Basic Brown 1	1,3-Benzenediamine	108-45-2
A	Basic Brown 1	1,2,4-Triaminobenzene	615-71-4
B	14408-20-9	2,6-Dichloro-4-nitroaniline	99-30-9
B	14408-20-9	Unknown 72	—
B	36986-04-6	2-Chloro-4-nitroaniline	121-87-9
B	36986-04-6	Unknown 72	—
B	59709-10-3	2-Chloro-4-nitroaniline	121-87-9
B	59709-10-3	Unknown 20	—
C	68929-07-7	2-(4-Amino-*N*-ethylanilino)ethanol	92-65-9
C	69852-41-1	2-(4-Amino-*N*-ethylanilino)ethanol	92-65-9
Individual 1	93783-70-1	*N*,*N*-Dimethyl-*p*-phenylenediamine	99-98-9
Individual 1	93783-70-1	Unknown 18	—
Individual 2	52769-39-8	Unknown 10	—
Individual 2	52769-39-8	Unknown 3	—

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index

Table C2: Genotoxicity and carcinogenicity of postulated azo bond cleavage products for Basic Orange 2, Basic Brown 1, Phenazopyridine hydrochloride and CAS RNs 63681-54-9 and 75660-25-2

Substance (C.I. name or CAS RN)	Postulated azo bond cleavage product (CAS RN)	Available data on genotoxicity and carcinogenicity of aromatic amine metabolite
Phenazopyridine hydrochloride Basic Orange 2 63681-54-9 75660-25-2	aniline 62-53-3	Carcinogenicity: Positive in male rats; negative in female rats and mice Genotoxicity: *In vivo,* some positive; *in vitro*, some positive (Health Canada 2011a)
Phenazopyridine hydrochloride	2,3,6-Triaminopyridine 4318-79-0	Carcinogenicity: No data Genotoxicity:

Basic Orange 2 63681-54-9 75660-25-2 Basic Brown 1	1,2,4-Triaminobenzene 615-71-4	Carcinogenicity: no data Genotoxicity: Mutagenic in two strains of *Salmonella typhimurium* that detect frameshift-inducing mutagens (Garner and Nutman 1977; Yoshikawa et al. 1979)
Basic Brown 1	1,3-Benzenediamine 108-45-2	Carcinogenicity: Equivocal pituitary adenoma and fibrosarcoma at injection site following subcutaneous injection; no relationship between exposure via oral, dermal or subcutaneous injection routes and carcinogenicity Genotoxicity: *In vivo*—equivocal (some positive, some negative); *in vitro*—mutagenicity (positive in Ames); equivocal clastogenicity

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index

Table C3: Genotoxicity and carcinogenicity of postulated azo bond cleavage products for subset B (CAS RNs 14408-20-9, 36986-04-6 and 59709-10-3), subset C (CAS RNs 68929-07-7 and 69852-41-1), CAS RN 93783-70-1 and CAS RN 52769-39-8

Substance (CAS RN)	**Postulated azo bond cleavage product (CAS RN)**	**Available data on genotoxicity and carcinogenicity of aromatic amine metabolite**
14408-20-9	99-30-9	Carcinogenicity: Negative in multiple cancer assays in experimental animals and in *in vivo* assays (FAO/WHO 1998) Genotoxicity: Mostly negative for *in vitro* genotoxicity assays (FAO/WHO 1998)
36986-04-6 59709-10-3	121-87-9	Genotoxicity: Positive for *in vitro* mutagenicity in bacteria and chromosomal aberration assay; negative *in vitro* for mutagenicity in mammalian cells, micronuclei and DNA damage and repair assays (Shimizu and Takemura 1984; Kawai et al. 1987; Yoshimi et al. 1988; Palus et al. 1995; JETOC 1996; Matsushima et al. 1999)
68929-07-7 69852-41-1	92-65-9	No data
93783-70-1	99-98-9	Carcinogenicity: No indication of carcinogenic potential (BG Chemie 1998). Genotoxicity: Positive for mutagenicity in bacteria and *in vitro* for chromosomal aberrations; weak positive in unscheduled DNA synthesis; negative for mutagenicity in bacteria and *in vitro* for chromosomal aberrations (Chung et al. 1978; Ashby et al. 1983; Kawalek et al. 1983; Kornbrust and Barfknecht 1984; Freeman et al. 1987; Sofuni et al. 1990; Zeiger et al. 1992; Galloway et al. 1997; Ben Mansour et al. 2009)

Abbreviation: CAS RN, Chemical Abstracts Service Registry Number

Table C4: Postulated azo bond cleavage products for the remaining Azo Basic Dyes

Substance (CAS RN)	**Postulated azo bond cleavage product (name)**	**Postulated azo bond cleavage product (CAS RN)**
2869-83-2	*N*,*N*-Dimethyl-*p*-phenylenediamine	99-98-9
4608-12-2	Phenazinium, 3-amino-7-(dimethylamino)-5-phenyl-, chloride	2390-56-9

4608-12-2	*N*,*N*-Dimethyl-*p*-phenylenediamine	99-98-9
4618-88-6	3,7-Diamino-5-phenylphenazin-5-ium chloride	81-93-6
4618-88-6	*N*,*N*-Dimethyl-*p*-phenylenediamine	99-98-9
10189-42-1	Pyridinium, 1-[2-(ethylphenylamino)ethyl]-, chloride	14408-19-6
10189-42-1	Benzenesulfonamide, 4-amino-3,5-dichloro-*N*,*N*-dimethyl-	17418-80-3
14970-39-9	1,4-Benzenediamine, *N*,*N*-diethyl-	93-05-0
23408-72-2	*N*,*N*-Dimethyl-*p*-phenylenediamine	99-98-9
23408-72-2	Unknown 76	N/A
29508-48-3	Unknown 67	N/A
29508-48-3	Unknown 68	N/A
63589-49-1	1,4-Benzenediamine, *N*,*N*-diethyl-	93-05-0
65150-98-3	1,4-Benzenediamine, *N*,*N*-diethyl-	93-05-0
68936-17-4	*N*,*N*-Dimethyl-*p*-phenylenediamine	99-98-9
72361-40-1	2-Bromo-4,6-dinitroaniline	1817-73-8
72361-40-1	Unknown 26	N/A
72379-36-3	Unknown 130	N/A
72379-36-3	Unknown 27	N/A
72379-37-4	Unknown 130	N/A
72379-37-4	Unknown 131	N/A
74744-63-1	Unknown 170	N/A
74744-63-1	Unknown 171	N/A
75199-20-1	*p*-Phenylenediamine	106-50-3
75199-20-1	Benzenamine, 4-(phenylazo)-	60-09-3
75199-20-1	Aniline	62-53-3
75199-20-1	Unknown 118	N/A
79234-33-6	1,2,4-Triaminobenzene	615-71-4
79234-33-6	Aniline	62-53-3
83969-13-5	*N*,*N*-Dimethyl-*p*-phenylenediamine	99-98-9
83969-13-5	Unknown 148	N/A
85114-37-0	Unknown 10	N/A
85114-37-0	Unknown 173	N/A
85480-88-2	Unknown 176	N/A
85480-88-2	Unknown 177	N/A
125329-01-3	*p*-Phenylenediamine	106-50-3
125329-01-3	Benzenamine, 4-(phenylazo)-	60-09-3
125329-01-3	Aniline	62-53-3

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index; N/A, not applicable

Appendix D: Azo Basic Dyes with Effects of Concern

Some of the Azo Basic Dyes in this assessment have effects of concern based on potential carcinogenicity. The details for supporting the potential carcinogenicity for these substances are outlined in section 7.2 Health Effects Assessment (see specific sub-sections), and generally based on one or more of the following lines of evidence:

- Classifications by national or international agencies for carcinogenicity (may be a group classification).
- Evidence of carcinogenicity in animal studies and/or human epidemiology based on the specific substance.
- Potential to release one or more of the EU22 aromatic amines by azo bond cleavage.
- Read-across to related substances for which one or more of the above lines of evidence apply.

Table D-1. Substances suspected of having effects of concern based on potential carcinogenicity.

Substance Name/acronym and CAS RN	Classification for carcinogenicity[b]	Evidence of carcinogenicity from animal studies and/or human epidemiology	Release of EU22 aromatic amine by azo bond cleavage	Read-across
Phenazopyridine hydrochloride 136-40-3	IARC 2B NTP "reasonably anticipated to be a human carcinogen"	X (NCI 1978)	-	-
Basic Orange 2 532-82-1 and related salts: 63681-54-9 (dodecyl-benzenesulfonate) 75660-25-2 (monoacetate) 79234-33-6 (acetate)	-	X (Albert 1956; IARC 1975. 1987b)	-	Read Across to phenazopyridine hydrochloride (See section 7.2.2)
1,3′-Bipyridinium, 1′,2′-dihydro-6′-hydroxy-3,4′-dimethyl-2′-oxo-5′-[[4-	-	-	*p*-aminoazobenzene	-

Substance Name/acronym and CAS RN	Classification for carcinogenicity[b]	Evidence of carcinogenicity from animal studies and/or human epidemiology	Release of EU22 aromatic amine by azo bond cleavage	Read-across
(phenylazo)phenyl] azo]-, chloride 75199-20-1 [a]				
Propanoic acid, 2-hydroxy-, compd. with 7-[[4,6-bis[[3-(diethylamino)propyl]amino]-1,3,5-triazin-2-yl]amino]-4-hydroxy-3-[[4-(phenylazo)phenyl] azo]-2-naphthalenesulfonic acid (1:1) 125329-01-3 [a]	-	-	*p*-aminoazobenzene	-

[a] There is uncertainty with respect to the extent of azo bond reduction and the actual metabolites released *in vivo*, in particular for a disazo substance with a postulated cleavage product that contains an azo bond.
[b] Classifications used for carcinogenicity are described in Environment Canada, Health Canada 2014b.