Screening Assessment

Aromatic Azo and Benzidine-based Substance Grouping

Certain Benzidine-based Dyes and Related Substances

Environment Canada
Health Canada

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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 9 Benzidine-based Acid Dyes, 24 Benzidine-based Direct Dyes, 2 Benzidine-based Cationic Indicators, 2 Benzidine-based Precursors and 5 Benzidine Derivatives. These 42 substances constitute 5 subgroups of the Aromatic Azo and Benzidine-based Substances 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 assessment as they met the categorization criteria under subsection 73(1) of CEPA 1999 and/or were considered as a priority based on other human health concerns.

The Chemical Abstracts Service Registry Number (CAS RN), *Domestic Substances List* (DSL) name, and Colour Index generic name or acronym of the 42 substances are presented in the following tables, by subgroup.

Identity of the 9 Benzidine-based Acid Dyes in the Aromatic Azo and Benzidine-based Substance Grouping

CAS RN	DSL name	Colour Index name or acronym
3701-40-4	2,7-Naphthalenedisulfonic acid, 4-hydroxy-3-[[4′-[(2-hydroxy-1-naphthalenyl)azo]-2,2′-dimethyl[1,1′-biphenyl]-4-yl]azo]	Acid Red 99
6459-94-5	1,3-Naphthalenedisulfonic acid, 8-[[3,3′-dimethyl-4′-[[4-[[(4-methylphenyl)sulfonyl]oxy]phenyl]azo][1,1′-biphenyl]-4-yl]azo]-7-hydroxy-, disodium salt	Acid Red 114
6470-20-8	[1,1′-Biphenyl]-2,2′-disulfonic acid, 4-[(4,5-dihydro-3-methyl-5-oxo-1-phenyl-1H-pyrazol-4-yl)azo]-4′-[(2-hydroxy-1-naphthalenyl)azo]-, disodium salt	Acid Orange 56
6548-30-7	1,3-Naphthalenedisulfonic acid, 8-[[3,3′-dimethoxy-4′-[[4-[[(4-methylphenyl)sulfonyl]oxy]phenyl]azo][1,1′-biphenyl]-4-yl]azo]-7-hydroxy-, disodium salt	Acid Red 128
68318-35-4	2,7-Naphthalenedisulfonic acid, 4-amino-3-[[4′-[(2,4-dihydroxyphenyl)azo]-3,3′-dimethyl[1,1′-biphenyl]-4-yl]azo]-5-hydroxy-6-[(4-sulfophenyl)azo]-, trisodium salt	Acid Black 209
68400-36-2	2,7-Naphthalenedisulfonic acid, 4-amino-5-hydroxy-6-[[4′-[(4-hydroxyphenyl)azo]-3,3′-dimethyl[1,1′-biphenyl]-4-yl]azo]-3-[(4-nitrophenyl)azo]-, disodium salt	NAAHD
83221-63-0	2,7-Naphthalenedisulfonic acid, 4-amino-3-[[4′-[(2,4-	NAADD

	diaminophenyl)azo]-2,2′-disulfo[1,1′-biphenyl]-4-yl]azo]-5-hydroxy-6-(phenylazo)-, sodium salt	
89923-60-4	Benzenesulfonic acid, 3,3′-[(2,2′-dimethyl[1,1′-biphenyl]-4,4′-diyl)bis[azo(4,5-dihydro-3-methyl-5-oxo-1H-pyrazole-4,1-	BADB
10169-02-5	[1,1′-Biphenyl]-2,2′-disulfonic acid, 4,4′-bis[(2-hydroxy-1-naphthalenyl)azo]-, disodium salt	Acid Red 97

Identity of the 24 Benzidine-based Direct Dyes in the Aromatic Azo and Benzidine-based Substance Grouping

CAS RN	DSL name	Colour Index name or acronym
72-57-1	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethyl[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-, tetrasodium salt	Direct Blue 14
573-58-0	1-Naphthalenesulfonic acid, 3,3′-[[1,1′-biphenyl]-4,4′-diylbis(azo)]bis[4-amino-, disodium salt	Direct Red 28
992-59-6	1-Naphthalenesulfonic acid, 3,3′-[(3,3′-dimethyl[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[4-amino-, disodium salt	Direct Red 2
2150-54-1	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethyl[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[4,5-dihydroxy-, tetrasodium salt	Direct Blue 25
2429-71-2	1-Naphthalenesulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[4-hydroxy-, disodium salt	Direct Blue 8
2429-74-5	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-, tetrasodium salt	Direct Blue 15
6420-06-0	1-Naphthalenesulfonic acid, 4-hydroxy-3-[[4′-[(1-hydroxy-5-sulfo-2-naphthalenyl)azo]-3,3′-dimethyl[1,1′-biphenyl]-4-yl]azo]-, disodium salt	Direct Violet 28
6420-22-0	2,7-Naphthalenedisulfonic acid, 5-amino-3-[[4′-[(6-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-3,3′-dimethyl[1,1′-biphenyl]-4-yl]azo]-4-hydroxy-, trisodium salt	Direct Blue 295
6449-35-0	1-Naphthalenesulfonic acid, 3-[[4′-[(6-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-3,3′-dimethoxy[1,1′′-biphenyl]-4-yl]azo]-4-hydroxy-, disodium salt	Direct Blue 151
6548-29-4	2,7-Naphthalenedisulfonic acid, 4,4′-[(3,3′-dichloro[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[3-amino-, tetrasodium salt	Direct Red 46
6655-95-4	Acetic acid, 2,2′-[[4,4′-bis[[1-hydroxy-6-[(4-	Direct Blue

CAS RN	DSL name	Colour Index name or acronym
	methoxyphenyl)amino]-3-sulfo-2-naphthalenyl]azo][1,1′-biphenyl]-3,3′-diyl]bis(oxy)]bis-, tetrasodium salt	158
16071-86-6	Cuprate(2−), [5-[[4′-[[2,6-dihydroxy-3-[(2-hydroxy-5-sulfophenyl)azo]phenyl]azo][1,1′-biphenyl]-4-yl]azo]-2-hydroxybenzoato(4-)]-, disodium	Direct Brown 95
67923-89-1	2,7-Naphthalenedisulfonic acid, 5-amino-4-hydroxy-3-[[4′-[(1-hydroxy-4-sulfo-2-naphthalenyl)azo]-3,3′-dimethoxy[1,1′-biphenyl]-4-yl]azo]-, trilithium salt	NAAH·3Li
70210-28-5	Benzoic acid, 5-[[4′-[[6-amino-5-(1H-benzotriazol-5-ylazo)-1-hydroxy-3-sulfo-2-naphthalenyl]azo]-3,3′-dimethoxy[1,1′-biphenyl]-4-yl]azo]-2-hydroxy-4-methyl-, disodium salt	BABHS
71215-83-3	Benzoic acid, 5-[[4′-[(2-amino-8-hydroxy-6-sulfo-1-naphthalenyl)azo]-2,2′-dichloro[1,1′-biphenyl]-4-yl]azo]-2-hydroxy-, disodium salt	BAHSD
71550-22-6	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-, tetralithium salt	NADB·4Li
72252-59-6	[1,1′-Biphenyl]-3,3′-dicarboxylic acid, 4-[[5-[[5-(aminosulfonyl)-2-hydroxyphenyl]azo]-1-hydroxy-6-(phenylamino)-3-sulfo-2-naphthalenyl]azo]-4′-[[1-[[(3-carboxy-4-hydroxyphenyl)amino]carbonyl]-2-oxopropyl]azo]-, tetrasodium salt	BDAAH
75659-72-2	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-, monolithium trisodium salt	NADB·Li·3Na
75659-73-3	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-, dilithium disodium salt	NADB·2Li·2Na
75673-18-6	2,7-Naphthalenedisulfonic acid, 5-amino-4-hydroxy-3-[[4′-[(1-hydroxy-4-sulfo-2-naphthalenyl)azo]-3,3′-dimethoxy[1,1′-biphenyl]-4-yl]azo]-, monolithium disodium salt	NAAH·Li·2Na
75673-19-7	2,7-Naphthalenedisulfonic acid, 5-amino-4-hydroxy-3-[[4′-[(1-hydroxy-4-sulfo-2-naphthalenyl)azo]-3,3′-dimethoxy[1,1′-biphenyl]-4-yl]azo]-, dilithium monosodium salt	NAAH·2Li·Na
75673-34-6	1-Naphthalenesulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[4-hydroxy-, dilithium salt	NADB·2Li
75673-35-7	1-Naphthalenesulfonic acid, 3,3′-[(3,3″-dimethoxy[1,1′-	NADB·Li·Na

CAS RN	**DSL name**	**Colour Index name or acronym**
	biphenyl]-4,4′-diyl)bis(azo)]bis[4-hydroxy-, monolithium monosodium salt	
75752-17-9	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-, trilithium monosodium salt	NADB·3Li·Na

Identity of the two Benzidine-based Cationic Indicators in the Aromatic Azo and Benzidine-based Substance Grouping

CAS RN	**DSL name**	**Colour Index name or acronym**
298-83-9	2H-Tetrazolium, 3,3′-(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis[2-(4-nitrophenyl)-5-phenyl-, dichloride	TDBPD
1871-22-3	2H-Tetrazolium, 3,3′-(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis[2,5-diphenyl-, dichloride	TDBD

Identity of the two Benzidine-based Precursors in the Aromatic Azo and Benzidine-based Substance Grouping

CAS RN	**DSL name**	**Colour Index name or acronym**
91-92-9	Naphthalenecarboxamide, *N*,*N*′-(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis[3-hydroxy-	Naphthol AS-BR
93940-21-7	1-Triazene-1-carbonitrile, 3,3′-(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis-	TCDB

Identity of the five Benzidine Derivatives in the Aromatic Azo and Benzidine-based Substance Grouping

CAS RN	**DSL name**	**Colour Index name or acronym**
91-97-4	1,1′-Biphenyl, 4,4′-diisocyanato-3,3′-dimethyl-	TODI
119-90-4	1,1′-Biphenyl]-4,4′-diamine, 3,3′-dimethoxy-	3,3′-DMOB
119-93-7*	1,1′-Biphenyl]-4,4′-diamine, 3,3′-dimethyl-	3,3′-DMB
366-29-0	[1,1′-Biphenyl]-4,4′-diamine, *N*,*N*,*N*′,*N*′-tetramethyl-	4N-TMB
612-82-8*	1,1′-Biphenyl]-4,4′-diamine, 3,3′-dimethyl-, dihydrochloride	3,3′-DMB·2HCl

* These substances were not identified under subsection 73(1) of CEPA 1999 but were included in this assessment as they were considered as priorities based on other human health concerns.

For the purpose of this Screening Assessment, four subgroups (Benzidine-based Acid Dyes, Direct Dyes, Cationic Indicators and Precursors) are collectively referred to as “Benzidine-based Substances” while Benzidine-based Acid Dyes and Benzidine-based Direct Dyes are collectively referred to as “Benzidine-based Dyes.”

The majority of the Benzidine-based Substances and Benzidine Derivatives in this grouping were not reported to be manufactured or imported in Canada in quantities above 100 kg/year according to recent surveys under section 71 of CEPA 1999.

Environment

Benzidine-based Dyes are anionic molecules that have relatively high water solubility (greater than 1 g/L) and are expected to dissociate at environmentally relevant pH levels. When considering potential releases of Benzidine-based Dyes to the environment, it is expected that the chemistry of these substances results in their partitioning to water, sediment and soil. Benzidine-based Dyes have low experimental log octanol–water partition coefficients (K_{ow}) and low fish bioconcentration factors (BCFs), indicating that these dyes are not likely to bioconcentrate in aquatic organisms. According to empirical and modelled data, Benzidine-based Dyes are expected to biodegrade very slowly in aerobic environments (water, sediment and soil); however, they may degrade and transform to certain benzidine derivatives if they reach anaerobic environments. Based on aquatic and terrestrial ecotoxicity data (including both empirical and read-across data), Benzidine-based Dyes have the potential to cause harm to aquatic and terrestrial organisms at low concentrations. A conservative aquatic exposure analysis of the textile wet processing sector and a conservative terrestrial exposure analysis of the application of biosolids to land were done because they were anticipated to present the highest potential ecological risks related to the industrial release of these substances. Predicted environmental concentrations (PECs) for Benzidine-based Dyes in aquatic and terrestrial environments were compared with predicted no-effect concentrations (PNECs) for each compartment, and calculated risk quotients were all below one. Considering all available lines of evidence with respect to the persistence, potential bioaccumulation, ecotoxicity, industrial uses and potential releases of the substances, it is concluded that Benzidine-based Dyes have a low potential to cause ecological harm in Canada.

Benzidine-based Cationic Indicators also have relatively high water solubility and are expected to dissociate at environmentally relevant pH levels. Given their high water solubility and affinity for negatively charged organic particles, potential releases would be expected to partition to water, sediment and soil. Since Benzidine-based Cationic Indicators are charged, they have low experimental log K_{ow} values. While BCFs were not available for these substances, they are similar enough in physical and chemical properties to Benzidine-based Dyes to suggest that they are not likely to bioconcentrate in aquatic organisms. Benzidine-based Cationic Indicators are expected to biodegrade very slowly in aerobic environments (water, sediment and soil); however, Benzidine-based Cationic Indicators may degrade and transform to certain benzidine derivatives if they reach anaerobic environments. Based on aquatic and terrestrial ecotoxicity data (including both empirical and read-across data), Benzidine-based Cationic Indicators have the potential to cause harm to aquatic organisms at low concentrations. However, considering all lines of evidence for these two Benzidine-based Cationic Indicators, particularly as no commercial activity in Canada was reported, it is believed that the substances would not present a risk to the environment. Therefore, it is concluded that these Benzidine-based Cationic Indicators have a low potential to cause ecological harm in Canada.

The Benzidine-based Precursors, Naphthol AS-BR and TCDB, were assessed individually, since their physical and chemical properties and chemical structures are very different. Models were used to predict most results, as no close analogues with relevant data could be found. Naphthol AS-BR is sparingly soluble and not expected to dissociate under environmentally relevant pH levels, so it is expected to reside mostly in soil or sediment if released to the environment. TCDB is moderately soluble and is expected to dissociate readily and stay in the water column or bind with particles, given its acidic character. Both Benzidine-based Precursors have moderate to high estimated log K_{ow} values and moderate to high modelled aquatic BCFs. Despite some tendency to bioaccumulate, modelled data show that these substances may metabolize significantly. These Benzidine-based Precursors are expected to biodegrade very slowly in aerobic environments (water, sediment and soil). Based on two sets of modelled aquatic toxicity data for acute and chronic endpoints, both TCDB and Naphthol AS-BR have the potential to cause harm to aquatic organisms at low concentrations (pivotal values < 1 mg/L). Considering all lines of evidence, including the fact that TCDB and Naphthol AS-BR are not known to be in commerce in Canada, it is believed that these substances would not present a risk to the environment. Therefore, it is concluded that these Benzidine-based Precursors have a low potential to cause ecological harm in Canada.

Benzidine Derivatives are generally moderately soluble, have low to moderate log K_{ow} values and will ionize at low pH levels. Based on these properties, as well as their high potential for binding to particulate matter and sediment, these substances are expected to be found in water, sediment and soil if released to the environment. Empirical and modelled data indicate that Benzidine Derivatives biodegrade slowly in aerobic environments (water, sediment and soil). Moderate to high water solubility, low to moderate log K_{ow} values as well as a low empirical BCF for 3,3′-DMB indicate that Benzidine Derivatives will not bioconcentrate in aquatic organisms. Empirical and modelled data indicate that Benzidine Derivatives are moderately to highly toxic to aquatic organisms. However, given that these substances do not have the potential to be released to the environment in high volumes, as they were not reported to be manufactured or imported in Canada in quantities above 100 kg/year, according to recent regulatory surveys; therefore, it is believed that Benzidine Derivatives would not present a risk to the environment. Therefore, it is concluded that these five Benzidine Derivatives have a low potential to cause ecological harm in Canada.

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 42 Benzidine-based Dyes and Related Substances evaluated in this assessment. It is concluded that these Benzidine-based Dyes and Related Substances 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.

Human Health

A critical effect for certain Benzidine-based Substances is potential carcinogenicity via reductive cleavage of the azo bonds and release of aromatic amines, which can be converted to reactive intermediates through metabolic oxidation. Therefore, Benzidine-based Substances are evaluated by examining their hazard potential (e.g., ability to undergo reductive cleavage and hazard potential of the corresponding aromatic amines), together with their potential for exposure of the general population of Canada. Similarly, the Benzidine Derivatives are evaluated for their hazard potential, together with their potential for exposure of the general population.

Exposure of the general population to Benzidine-based Substances and derivatives from environmental media is not expected due to limited commercial quantities in Canada. Therefore, risk to human health from these sources is not expected.

Available information indicates that residual 3,3′-DMB may leach into foods being prepared with polyamide cooking utensils. Margins between the estimated daily oral exposure from use of polyamide cooking utensils and critical effect levels are considered adequate to address uncertainties in the health effects and exposure databases.

The Benzidine Derivatives 3,3′-DMB and 3,3′-DMOB are regulated in textiles in the European Union. Two European surveys, as well as a Japanese study, indicate that 3,3′-DMB and 3,3′-DMOB are occasionally detected in some textiles and leather products, some of which were reported to be imported from other countries. Accordingly, these two Benzidine Derivatives could be present in imported products in Canada, as the Canadian textile market is predominantly composed of imported products. Testing of products on the Canadian market, however, did not identify these two Benzidine Derivatives in imported and domestic textile and leather products. Exposure to 3,3′-DMB and 3,3′-DMOB from textiles and leather is considered to be limited; direct and prolonged skin contact is not expected. Therefore, risk to human health is not expected.

Exposure of the general population to the other three Benzidine Derivatives (3,3′-DMB·2HCl, TODI and 4N-TMB) is not expected. Therefore, risk to human health is not expected.

Three of the five Benzidine Derivatives in this assessment (3,3′-DMB, 3,3′-DMB·2HCl and 3,3′-DMOB) have human health effects of concern based on their potential carcinogenicity.

Potential for general population exposure due to dermal contact with textile clothing and leather articles was identified for one of the Benzidine-based Acid Dyes, Acid Red 97. Although exposure to Acid Red 97 is expected for the general population, the hazard potential, based on available information, is considered low. Accordingly, the risk to human health is considered to be low.

For the remaining Benzidine-based Acid Dyes in this assessment, and all of the benzidine-based Direct Dyes in this assessment, available information (including surveys conducted under section 71 of *CEPA 1999* and information on uses pursuant to the *Food and Drugs Act*) indicates that exposure from textiles and leather is considered to be limited; direct and prolonged skin contact is not expected. Therefore, risk to human health is not expected.

Exposure of the general population to the two Benzidine-based Precursors and two Benzidine-based Cationic Indicators in this assessment is not expected. Therefore, risk to human health is not expected.

Some of the Benzidine-based Acid Dyes, Benzidine-based Direct Dyes, and Benzidine-based Precursors in this assessment have human health effects of concern based on potential carcinogenicity. These include the Benzidine-based Substances which may release benzidine, 3,3′-dichlorobenzidine (3,3′-DCB), 3,3′-DMB, 3,3′-DMOB, 2,2′-dimethylbenzidine (2,2′-DMB) or 2,2′-dichlorobenzidine (2,2′-DCB).

Based on the information presented in this Screening Assessment, it is concluded that the Benzidine-based Dyes and Related Substances 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.

Overall Conclusion

It is concluded that the Benzidine-based Dyes and Related Substances 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 sections 68 or 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 to determine whether these substances present or may present a risk to the environment or to human health.

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, significant 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 Benzidine-based Acid Dyes, Benzidine-based Direct Dyes, Benzidine-based Cationic Indicators, and Benzidine-based Precursors, and Benzidine Derivatives subgroups. Consideration of azo bond cleavage products (aromatic amines) is a key element of human health assessment in each subgroup. Some aromatic amines, commonly referred to as EU22 aromatic amines[1], 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).

Ten Benzidine-based Acid Dyes and 25 Benzidine-based Direct Dyes originally constituted 2 subgroups of the Aromatic Azo and Benzidine-based Substance Grouping.

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[1] Twenty-two aromatic amines listed in Appendix 8 of Regulation (EC) No. 1907/2006 (EU 22).

One Benzidine-based Acid Dye (Acid Red 111) and one Benzidine-based Direct Dye (Direct Black 38) were previously assessed during the Challenge Initiative of the CMP. It was concluded that Acid Red 111 and Direct Black 38 did not meet the criteria under section 64 of CEPA 1999 (Environment Canada, Health Canada 2009, 2011). Similarly, two of the Benzidine-based Acid Dyes (NAAHD and Acid Red 99), one of the Benzidine-based Direct Dyes (Direct Violet 28), and one of the Benzidine-based Cationic Indicators (TDBD) were previously included as part of a screening assessment, in April 2008, of 145 persistent, bioaccumulative, and inherently toxic (PBiT) substances that were considered not to be in commerce. No significant new information has been identified for Acid Red 111 or Direct Black 38, and therefore these substances are not included in the current Screening Assessment. However, Acid Red 111 and Direct Black 38 are used in this report for read-across purposes due to their structural similarity to the other Benzidine-based Acid Dyes and Benzidine-based Direct Dyes in these subgroups. In contrast, NAAHD, Acid Red 99, Direct Violet 28, and TDBD are included in the Benzidine-based Substances considered in this Screening Assessment because significant new information has been identified.

Information on Benzidine and 3,3′-dichlorobenzidine (3,3′-DCB) was used to inform this Screening Assessment. These two benzidine derivatives were previously assessed under the Priority Substances List (PSL) program and are listed on Schedule 1 (List of Toxic Substances) under CEPA 1999 (Canada 1993a, 1993b). Benzidine is regulated under the *Prohibition of Certain Toxic Substances Regulation*, 2012 (Canada 2012).

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.[2]

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 May 2014. Empirical data from key studies as well as some results from models were used to

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

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

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

The Screening Assessment was prepared by staff in the Existing Substances Programs at Health Canada and Environment Canada and incorporates input from other programs within these departments. The ecological and human health portions of this assessment have undergone external written peer review and 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 (University of Montreal, Canada). Additionally, the draft of this Screening Assessment was subject to a 60-day public comment period. While external comments were taken into consideration, the final content and outcome of the Screening 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 42 substances that belong to 5 subgroups that are part of the Aromatic Azo and Benzidine-based Substance Grouping. These subgroups are based on structural similarity and similar applications: Benzidine-based Acid Dyes (9 substances), Benzidine-based Direct Dyes (24 substances), Benzidine-based Cationic Indicators (2 substances), Benzidine-based Precursors (2 substances) and Benzidine Derivatives (5 substances) (Environment Canada and Health Canada. 2013). Two substances, Acid Red 111 and Direct Black 38, which were previously assessed in the Challenge Initiative, are included to inform the assessment.

For the purpose of this Screening Assessment, four subgroups (Benzidine-based Acid Dyes, Direct Dyes, Cationic Indicators and Precursors) are collectively referred to as "Benzidine-based Substances" while Benzidine-based Acid Dyes and Benzidine-based Direct Dyes are collectively referred to as "Benzidine-based Dyes."

The identities of the individual substances in this Screening Assessment are presented in Tables 2-1 to 2-5. The CAS RNs, *Domestic Substances List* (DSL) names, Colour Index (C.I.) generic names, and chemical acronyms of these substances are presented in Tables 2-1 to 2-5. Chemical acronyms are derived from the C.I. generic names when available; otherwise, they are based on the DSL names. A list of additional chemical names (e.g., trade names) is available from the National Chemical Inventories (NCI 2007).

Table 2-1. Identity of the Benzidine-based Acid Dyes

CAS RN	DSL name	C.I. generic name	Chemical acronym
3701-40-4	2,7-Naphthalenedisulfonic acid, 4-hydroxy-3-[[4′-[(2-hydroxy-1-naphthalenyl)azo]-2,2′-dimethyl[1,1′-biphenyl]-4-yl]azo]	Acid Red 99	N/A
6459-94-5	1,3-Naphthalenedisulfonic acid, 8-[[3,3′-dimethyl-4′-[[4-[[(4-methylphenyl)sulfonyl]oxy]phenyl]azo][1,1′-biphenyl]-4-yl]azo]-7-hydroxy-, disodium salt	Acid Red 114	N/A
6470-20-8	[1,1′-Biphenyl]-2,2′-disulfonic acid, 4-[(4,5-dihydro-3-methyl-5-oxo-1-phenyl-1H-pyrazol-4-yl)azo]-4′-[(2-hydroxy-1-naphthalenyl)azo]-, disodium salt	Acid Orange 56	N/A

CAS RN	DSL name	C.I. generic name	Chemical acronym
6548-30-7	1,3-Naphthalenedisulfonic acid, 8-[[3,3′-dimethoxy-4′-[[4-[[(4-methylphenyl)sulfonyl]oxy]phenyl]azo][1,1′-biphenyl]-4-yl]azo]-7-hydroxy-, disodium salt	Acid Red 128	N/A
10169-02-5	[1,1′-Biphenyl]-2,2′-disulfonic acid, 4,4′-bis[(2-hydroxy-1-naphthalenyl)azo]-, disodium salt	Acid Red 97	N/A
68318-35-4	2,7-Naphthalenedisulfonic acid, 4-amino-3-[[4′-[(2,4-dihydroxyphenyl)azo]-3,3′-dimethyl[1,1′-biphenyl]-4-yl]azo]-5-hydroxy-6-[(4-sulfophenyl)azo]-, trisodium salt	Acid Black 209	N/A
68400-36-2	2,7-Naphthalenedisulfonic acid, 4-amino-5-hydroxy-6-[[4′-[(4-hydroxyphenyl)azo]-3,3′-dimethyl[1,1′-biphenyl]-4-yl]azo]-3-[(4-nitrophenyl)azo]-, disodium salt	NA	NAAHD
83221-63-0	2,7-Naphthalenedisulfonic acid, 4-amino-3-[[4′-[(2,4-diaminophenyl)azo]-2,2′-disulfo[1,1′-biphenyl]-4-yl]azo]-5-hydroxy-6-(phenylazo)-, sodium salt	NA	NAADD
89923-60-4	Benzenesulfonic acid, 3,3′-[(2,2′-dimethyl[1,1′-biphenyl]-4,4′-diyl)bis[azo(4,5-dihydro-3-methyl-5-oxo-1H-pyrazole-4,1-	NA	BADB

Abbreviations: N/A, not applicable (chemical acronym); NA, not available (C.I.generic name)

Table 2-2. Identity of the Benzidine-based Direct Dyes

CAS RN	DSL name	C.I. generic name	Chemical acronym
72-57-1	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethyl[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-, tetrasodium salt	Direct Blue 14	N/A
573-58-0	1-Naphthalenesulfonic acid, 3,3′-[[1,1′-biphenyl]-4,4′-diylbis(azo)]bis[4-amino-, disodium salt	Direct Red 28	N/A
992-59-6	1-Naphthalenesulfonic acid, 3,3′-[(3,3′-dimethyl[1,1′-biphenyl]-4,4′-	Direct Red 2	N/A

CAS RN	DSL name	C.I. generic name	Chemical acronym
	diyl)bis(azo)]bis[4-amino-, disodium salt		
2150-54-1	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethyl[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[4,5-dihydroxy-, tetrasodium salt	Direct Blue 25	N/A
2429-71-2	1-Naphthalenesulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[4-hydroxy-, disodium salt	Direct Blue 8	N/A
2429-74-5	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-, tetrasodium salt	Direct Blue 15	N/A
6420-06-0	1-Naphthalenesulfonic acid, 4-hydroxy-3-[[4′-[(1-hydroxy-5-sulfo-2-naphthalenyl)azo]-3,3′-dimethyl[1,1′-biphenyl]-4-yl]azo]-, disodium salt	Direct Violet 28	N/A
6420-22-0	2,7-Naphthalenedisulfonic acid, 5-amino-3-[[4′-[(6-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-3,3′-dimethyl[1,1′-biphenyl]-4-yl]azo]-4-hydroxy-, trisodium salt	Direct Blue 295	N/A
6449-35-0	1-Naphthalenesulfonic acid, 3-[[4′-[(6-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-3,3′-dimethoxy[1,1′-biphenyl]-4-yl]azo]-4-hydroxy-, disodium salt	Direct Blue 151	N/A
6548-29-4	2,7-Naphthalenedisulfonic acid, 4,4′-[(3,3′-dichloro[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[3-amino-, tetrasodium salt	Direct Red 46	N/A
6655-95-4	Acetic acid, 2,2′-[[4,4′-bis[[1-hydroxy-6-[(4-methoxyphenyl)amino]-3-sulfo-2-naphthalenyl]azo][1,1′-biphenyl]-3,3′-diyl]bis(oxy)]bis-, tetrasodium salt	Direct Blue 158	N/A
16071-86-6	Cuprate(2−), [5-[[4′-[[2,6-dihydroxy-3-[(2-hydroxy-5-sulfophenyl)azo]phenyl]azo][1,1′-biphenyl]-4-yl]azo]-2-	Direct Brown 95	N/A

CAS RN	DSL name	C.I. generic name	Chemical acronym
	hydroxybenzoato(4-)]-, disodium		
67923-89-1	2,7-Naphthalenedisulfonic acid, 5-amino-4-hydroxy-3-[[4′-[(1-hydroxy-4-sulfo-2-naphthalenyl)azo]-3,3′-dimethoxy[1,1′-biphenyl]-4-yl]azo]-, trilithium salt	NA	NAAH·3Li
70210-28-5	Benzoic acid, 5-[[4′-[[6-amino-5-(1H-benzotriazol-5-ylazo)-1-hydroxy-3-sulfo-2-naphthalenyl]azo]-3,3′-dimethoxy[1,1′-biphenyl]-4-yl]azo]-2-hydroxy-4-methyl-, disodium salt	NA	BABHS
71215-83-3	Benzoic acid, 5-[[4′-[(2-amino-8-hydroxy-6-sulfo-1-naphthalenyl)azo]-2,2′-dichloro[1,1′-biphenyl]-4-yl]azo]-2-hydroxy-, disodium salt	NA	BAHSD
71550-22-6	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-, tetralithium salt	NA	NADB·4Li
72252-59-6	[1,1′-Biphenyl]-3,3′-dicarboxylic acid, 4-[[5-[[5-(aminosulfonyl)-2-hydroxyphenyl]azo]-1-hydroxy-6-(phenylamino)-3-sulfo-2-naphthalenyl]azo]-4′-[[1-[[(3-carboxy-4-hydroxyphenyl)amino]carbonyl]-2-oxopropyl]azo]-, tetrasodium salt	NA	BDAAH
75659-72-2	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-, monolithium trisodium salt	NA	NADB·Li·3Na
75659-73-3	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-, dilithium disodium salt	NA	NADB·2Li·2Na
75673-18-6	2,7-Naphthalenedisulfonic acid, 5-amino-4-hydroxy-3-[[4′-[(1-hydroxy-4-sulfo-2-naphthalenyl)azo]-3,3′-dimethoxy[1,1′-biphenyl]-4-yl]azo]-, monolithium disodium salt	NA	NAAH·Li·2Na
75673-19-7	2,7-Naphthalenedisulfonic acid, 5-	NA	NAAH·2Li·Na

CAS RN	DSL name	C.I. generic name	Chemical acronym
	amino-4-hydroxy-3-[[4′-[(1-hydroxy-4-sulfo-2-naphthalenyl)azo]-3,3′-dimethoxy[1,1′-biphenyl]-4-yl]azo]-, dilithium monosodium salt		
75673-34-6	1-Naphthalenesulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[4-hydroxy-, dilithium salt	NA	NADB·2Li
75673-35-7	1-Naphthalenesulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[4-hydroxy-, monolithium monosodium salt	NA	NADB·Li·Na
75752-17-9	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-, trilithium monosodium salt	NA	NADB·3Li·Na

Abbreviations: N/A, not applicable; NA, not available (C.I.generic name)

Table 2-3. Identity of the Benzidine-based Cationic Indicators

CAS RN	DSL name	C.I. generic name	Chemical acronym
298-83-9	2H-Tetrazolium, 3,3′-(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis[2-(4-nitrophenyl)-5-phenyl-, dichloride	N/A	TDBPD
1871-22-3	2H-Tetrazolium, 3,3′-(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis[2,5-diphenyl-, dichloride	N/A	TDBD

Abbreviations: N/A, not applicable; NA, not available (C.I.generic name)

Table 2-4. Identity of the Benzidine-based Precursors

CAS RN	DSL name	C.I. generic name	Chemical acronym
91-92-9	Naphthalenecarboxamide, *N*,*N*′-(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis[3-hydroxy-	N/A	Naphthol AS-BR
93940-21-7	1-Triazene-1-carbonitrile, 3,3′-(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis-	N/A	TCDB

Abbreviations: N/A, not applicable; NA, not available (C.I.generic name)

Table 2-5. Identity of the Benzidine Derivatives

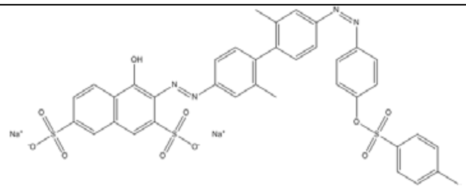
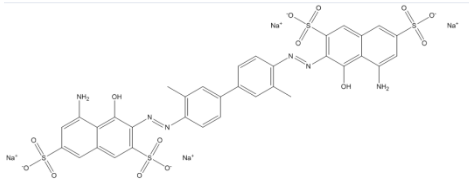
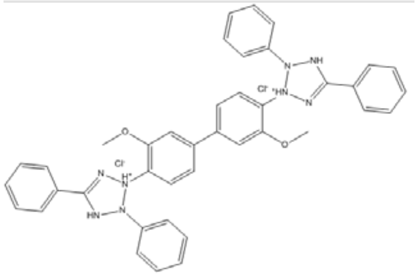
CAS RN	DSL name	C.I. generic name	Chemical acronym
91-97-4	1,1′-Biphenyl, 4,4′-diisocyanato-3,3′-dimethyl-	N/A	TODI
119-90-4	1,1′-Biphenyl]-4,4′-diamine, 3,3′-dimethoxy-	N/A	3,3′-DMOB
119-93-7	1,1′-Biphenyl]-4,4′-diamine, 3,3′-dimethyl-	N/A	3,3′-DMB
366-29-0	[1,1′-Biphenyl]-4,4′-diamine, *N*,*N*,*N*′,*N*′-tetramethyl-	N/A	4N-TMB
612-82-8	1,1′-Biphenyl]-4,4′-diamine, 3,3′-dimethyl-, dihydrochloride	N/A	3,3′-DMB·2HCl

Abbreviations: N/A, not applicable; NA, not available (C.I.generic name)

Example chemical structures, molecular formulas, group descriptions and molecular weight ranges are presented in Table 2-6 for the subgroups of Benzidine-based Acid Dyes, Benzidine-based Direct Dyes and Benzidine-based Cationic Indicators. Due to overall chemical similarity, including common functional groups (as described in Table 2-6), sharing similar physical and chemical properties and lack of data for individual substances, data for these three application classes (for which groupings are based on the method and conditions of their use) will be used for ecological exposure estimation approaches (when warranted). This general approach is discussed further in the section below on selection of analogues for read-across. Despite their similarities, these substances may have different potential transformation products. This is discussed further in the Environmental Persistence section and in the Human Health Effects section. Individual chemical structures, molecular formulas and molecular weights are presented for all Benzidine-based Dyes and Benzidine-based Cationic Indicators in Appendix A, Tables A1 – A3.

Table 2-6. Example structures and descriptions for Benzidine-based Dyes and Benzidine-based Cationic Indicators

Subgroup	Example structure for the subgroup	Group description with critical functional groups	Molecular weight range (g/mol)

Subgroup	**Example structure for the subgroup**	**Group description with critical functional groups**	**Molecular weight range (g/mol)**
Benzidine-based Acid Dyes (*n* = 10)	 $C_{37}H_{30}N_4O_{10}S_3Na_2$ (Acid Red 111)	Benzidine fragment, 1–2 naphthalene groups, 2–3 azo groups, 2–4 sulfonic acid groups, 3–4 benzene rings	699–920
Benzidine-based Direct Dyes (*n* = 25)	 $C_{34}H_{24}N_6O_{14}S_4Na_4$ (Direct Blue 14)	Benzidine fragment, 0–2 naphthalene groups, 2–3 azo groups, 1–4 sulfonic acid groups, 2–5 benzene rings, 0–3 amino groups, 0–1 benzotriazol groups	696–1134
Benzidine-based Cationic Indicators (*n* = 2)	 $C_{40}H_{30}N_8O_2$ (TDBD)	Benzidine fragment, 2 tetrazoliums, 6 benzene rings	655–818

Specific identities are presented for each of the Benzidine-based Precursors (Table 2-7) and Benzidine Derivatives (Table 2-8), given their more diverse chemical structure, physical and chemical properties.

Table 2-7. Structures and descriptions for specific Benzidine-based Precursors

Chemical name	**Chemical structure**	**Substance description with**	**Molecular weight**

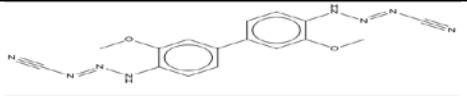
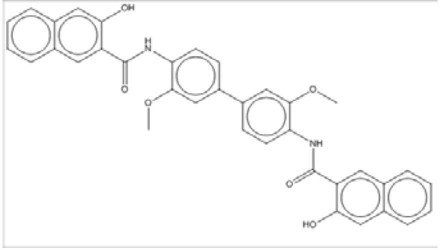
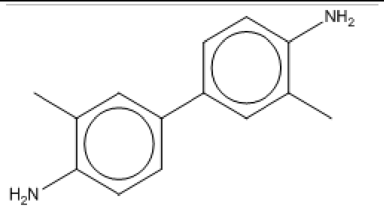
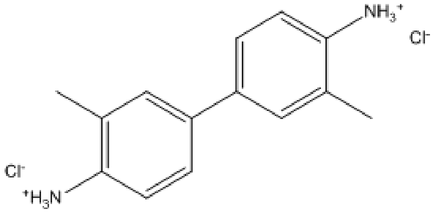
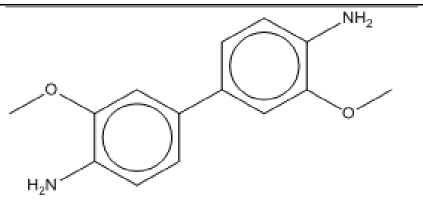
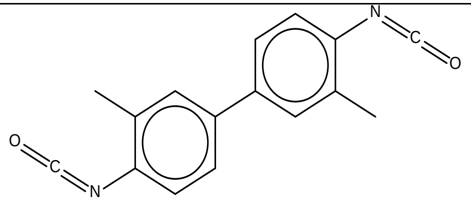
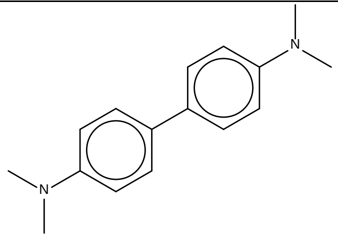
		critical functional groups	**(g/mol)**
TCDB	 $C_{16}H_{14}N_8O_2$	Benzidine fragment, 2 azo groups, 2 nitrile groups	351
Naphthol AS-BR	 $C_{36}H_{28}N_2O_6$	Benzidine fragment, 2 naphthol groups	585

Table 2-8. Structures and descriptions for specific Benzidine Derivatives

Chemical name	**Chemical structure**	**Substance description with critical functional groups**	**Molecular weight (g/mol)**
3,3′-DMB	 $C_{14}H_{16}N_2$	Benzidine fragment	212
3,3′-DMB·2HCl	 $C_{14}H_{18}CL_2N_2$	Benzidine fragment, 2 chlorine ions	285

Chemical name	Chemical structure	Substance description with critical functional groups	Molecular weight (g/mol)
3,3′-DMOB	 $C_{14}H_{16}N_2O_2$	Benzidine fragment	244
TODI	 $C_{16}H_{12}N_2O_2$	Biphenyl fragment, 2 isocyanate moieties	264
4N-TMB	 $C_{16}H_{20}N_2$	Benzidine fragment, 2 tertiary amines	240

In general, Benzidine-based Direct Dyes are of higher molecular mass than the Benzidine-based Acid Dyes and Benzidine-based Cationic Indicators, while Benzidine Derivatives and the two Benzidine-based Precursors have much lower molecular masses, since most can be used to synthesize dyes or pigments. Acid and direct dyes may each contain one or more azo, naphthalene and sulfonic acid groups and have 2–5 benzene rings each. The Benzidine-based Direct Dyes in this report may also contain amino groups that the Benzidine-based Acid Dyes do not. The two Benzidine-based Cationic Indicators each contain two tetrazolium moieties.

2.1 Selection of Analogues 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). 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 Human Health Effects Assessment section of this report.

Analogues used to inform the ecological assessment were selected based on the availability of relevant empirical data pertaining to physical and chemical properties and aquatic ecotoxicity data for the Benzidine-based Dyes as well as the two Benzidine-based Cationic Indicators were identified from within each respective group of substances contained in this Screening Assessment. A few additional analogue data points from non-benzidine-based acid dyes (Acid Yellow 23, Acid Yellow 36, Acid Orange 7; Table 2-9) were also considered for the Benzidine-based Acid Dyes to contribute to the weight of evidence. Analogue data for the Benzidine-based Cationic Indicators were also identified from outside the Benzidine-based Cationic Indicators application class. Basic Brown 4, an azo-based cationic dye (Table 2-9), and a tetrazolium substance (2,3,5-triphenyltetrazolium chloride, or TTC; Table 2-9) provide read-across data for ecotoxicity. The US Environmental Protection Agency (US EPA), in its chemical categories document, notes that no relationship between structure and activity has been found for cationic dyes (US EPA 2010), which are similar to the Benzidine-based Cationic Indicators. That being said, some categories of basic dyes (e.g., triarylmethanes) have been found to share certain characteristics, such as high toxicity. Given a lack of available empirical information and an acceptable level of similarity based on physical and chemical properties as well as the environmental fate of soluble Benzidine-based Acid and Direct Dyes and Benzidine-based Cationic Indicators, bioaccumulation and soil toxicity data are shared across these three subgroups.

Analogue data for one of the Benzidine-based Precursors (Naphthol AS-BR) were also identified from a substance outside the application classes identified in this assessment (3-hydroxy-*N*-phenyl-2-naphthalenecarboxamide, or NHNP; Table 2-9).

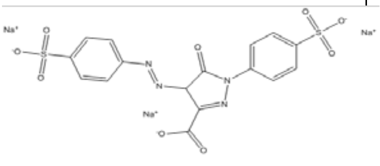
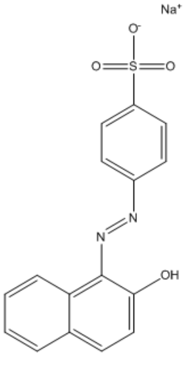
Acceptable analogue candidates were identified within the Benzidine Derivatives group for four of the five substances. The substance 3,3′-DMB will be used as an analogue for 3,3′-DMB·2HCl and TODI for physical and chemical properties, persistence, bioaccumulation and ecotoxicity. 3,3′-DMB·2HCl is in a crystalline form that behaves as a salt and will release 3,3′-DMB when in solution. TODI rapidly hydrolyzes in water to its associated amine, 3,3′-DMB, also referred to as TODA. 3,3′-DMB will also be used as an analogue for 3,3′-DMOB to evaluate its bioaccumulation potential and ecotoxicity, since no empirical data are available. These substances are similar, with the exception of methoxy groups in the *ortho* position to the amines for 3,3′-DMOB instead of methyl groups for 3,3′-DMB. Therefore, it is expected that the substances will have similar bioavailability and mode of action.

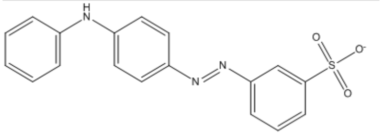
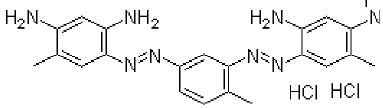
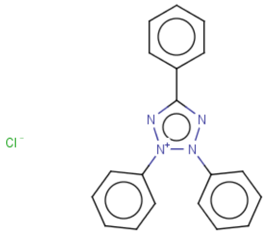
Benzidine (CAS RN 92-87-5; Table 2-9) was selected as a suitable analogue for 4N-TMB to correct some physical and chemical property estimates. 4N-TMB is structurally similar to benzidine, with the difference that it is a tertiary amine, containing two methyl substituents on each nitrogen atom, thereby missing the amine component (–NH–) that could undergo *N*-hydroxylation.

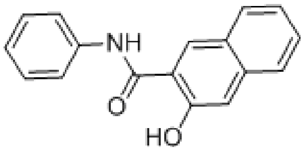
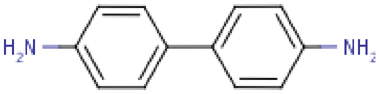
Analogue data are very important to this Screening Assessment, since the physical and chemical properties of many dyes are not amenable to model prediction, as they are considered to be out of the model domain of applicability (e.g., structural and/or parametric domains).

Therefore, the applicability of (Q)SAR models to dyes is determined on a case-by-case basis. In some cases, empirical analogue data will be adjusted to account for structural differences between the substance being assessed and the analogue using the Experimental Value Adjustment (EVA) method in EPISuite version 4.1 (2012).

Table 2-9. Identity of analogues and parameters to be used to inform the physical and chemical properties, environmental fate and potential to cause ecological harm

Name (acronym)	Chemical structure and formula (CAS RN)	Molecular weight (g/mol)	Parameters to be used in report	Description
Acid Yellow 23	 $C_{16}H_8N_4O_9S_2Na_4$ (1934-21-0)	556.34	K_{ow} Read-across for Benzidine-based Acid Dyes	AY23 is a monoazo acid dye. It differs in that it lacks the benzidine moiety and has a heterocyclic nitrogen moiety.
Acid Orange 7		350.33	K_{ow} Read-across for Benzidine-based Acid Dyes	AO7 is a monoazo acid dye. It differs in that it has a lower molecular mass and lacks the benzidine moiety.

Name (acronym)	**Chemical structure and formula (CAS RN)**	**Molecular weight (g/mol)**	**Parameters to be used in report**	**Description**
	$C_{16}H_{11}N_2O_4SNa$ (633-96-5)			
Acid Yellow 36	 $C_{18}H_{14}N_3O_3SNa$ (587-98-4)	375.38	K_{ow} Read-across for Benzidine-based Acid Dyes	AY36 is a monoazo acid dye. It differs in that it has a lower molecular mass and lacks the benzidine moiety.
Basic Brown 4	 $C_{21}H_{26}Cl_2N_8$ (5421-66-9)	461	Ecotoxicity Read-across for Benzidine-based Cationic Indicators	BB4 is a water-soluble cationic dye with two chloride ions. It differs in that it is a *meta*-phenylenediamine instead of benzidine based and has a lower molecular mass.
2,3,5-Triphenyltetrazolium chloride (TTC)	 $C_{19}H_{15}CLN_4$ (298-96-4)	335	Ecotoxicity Read-across for Benzidine-based Cationic Indicators	TTC contains the tetrazolium chloride moiety, which is almost exactly one-half of the structure of the two Benzidine-based Cationic Indicators.
2-Naphthalenecarboxamide, 3-		263	Melting point	This is about one half of the structure of

Name (acronym)	**Chemical structure and formula (CAS RN)**	**Molecular weight (g/mol)**	**Parameters to be used in report**	**Description**
hydroxy-*N*-phenyl- (NHNP)	 $C_{36}H_{28}N_2O_6$ (92-77-3)		Read-across for Naphthol AS-BR (Benzidine-based Precursor)	Naphthol AS-BR.
Benzidine	 $C_{12}H_{12}N_2$ (92-87-5)	184	Henry's Law constant, water solubility and K_{ow} Read-across to correct estimates for five Benzidine Derivatives	Benzidine is the critical fragment for all Benzidine Derivatives.

Abbreviations: 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.

A subset of physical and chemical properties of Benzidine-based Acid Dyes, Benzidine-based Direct Dyes and Benzidine-based Cationic Indicators—namely, melting point, water solubility and log octanol–water partition coefficient (log K_{ow})—is important in terms of ecological and human health assessment. A summary of the experimental physical and chemical properties of substances in the Benzidine-based Dyes and Benzidine-based Cationic Indicators subgroups that are relevant to their environmental fate and ecotoxicity is presented in Table 3-1. Pivotal values, including either single mean data points (e.g., melting point and decomposition) or a range of values, have been chosen to represent the properties of each subgroup. Detailed substance-specific information on these substances can be found in Tables A4–A6 in Appendix A of this report.

All Benzidine-based Dyes and Benzidine-based Cationic Indicators in this assessment are relatively large (molecular weights > 600 g/mol), complex ionic molecules (acid and direct dyes are anionic molecules and cationic indicators are cationic molecules), tend to dissociate at environmentally relevant pH levels and are highly soluble in water (in excess of 1 g/L) due to the presence of solubilizing functional groups (Hunger 2003; Table 3-1). Benzidine-based Acid Dyes are sodium salts that contain solubilizing sulfonic acid groups, while Benzidine-based Cationic Indicators contain tetrazolium functional groups with solubilizing positive charges. Given their hydrophilicity and ionic character, as demonstrated by low pK_a values (an indicator of acid dissociation), Benzidine-based Acid and Direct Dyes tend to have very low experimental log K_{ow} values (Table 3-1).

While no quantitative data are available for the two Benzidine-based Cationic Indicators, they are basic substances that are known to dissociate in water and have low log K_{ow} values (Øllgaard et al. 1998).

While experimental data are limited, all Benzidine-based Dyes and Benzidine-based Cationic Indicators are expected to have very low vapour pressures and very low Henry’s Law constant values (Øllgaard et al. 1998).

Table 3-1. A summary of experimental physical and chemical properties (at standard temperature of approximately 25°C) for the Benzidine-based Acid Dyes, Benzidine-based Direct Dyes and Benzidine-based Cationic Indicators

Subset	Property	Value(s) or range (for more than 3 data points)	Pivotal value(s) used in this assessment (basis for selection)
Benzidine-based Acid Dyes	Melting point and decomposition (°C)	170; 185; 190	182 (mean)
Benzidine-based Acid Dyes	Water solubility (mg/L)	> 500–65 000 (*n* = 4)	> 500–65 000 (range is used)
Benzidine-based Acid Dyes	Log K_{ow} (dimensionless)	−0.017; 0.57; 0.7	0.42 (mean)
Benzidine-based Acid Dyes	Effective minimum cross-sectional diameter (D_{min}, nm)	0.96–1.15 (*n* = 10)	Range used and discussed in text
Benzidine-based Acid Dyes	Effective maximum cross-sectional diameter (D_{max}, nm)	1.27–1.78 (*n* = 10)	Range used and discussed in text
Benzidine-based Acid Dyes	pK_a (dimensionless)	pK_{a1} = −0.67 pK_{a2} = −0.23	(sole pK_a estimates)
Benzidine-based Direct Dyes	Melting point and decomposition (°C)	109 – > 360 (*n* = 6)	> 317 (mean)
Benzidine-based Direct Dyes	Water solubility (mg/L)	1000–116 000 (*n* = 4)	1000–116 000 (range is used)
Benzidine-based Direct Dyes	Log K_{ow} (dimensionless)	0.77	0.77 (sole value)
Benzidine-based Direct Dyes	Effective minimum cross-sectional diameter (D_{min}, nm)	0.95–1.27 (*n* = 25)	Range used and discussed in text
Benzidine-based Direct Dyes	Effective maximum cross-sectional diameter	1.24–2.07 (*n* = 25)	Range used and discussed in text

Subset	Property	Value(s) or range (for more than 3 data points)	Pivotal value(s) used in this assessment (basis for selection)
	(D_{max}, nm)		
Benzidine-based Direct Dyes	pK_a (dimensionless)	$pK_{a1} = -1.5$ $pK_{a2} = -1.3$	(sole pK_a estimates)
Benzidine-based Cationic Indicators	Melting point and decomposition (°C)	189–255 (*n* = 4)	209 (mean)
Benzidine-based Cationic Indicators	Water solubility (mg/L)	9000, 10 000	9500 (mean)
Benzidine-based Cationic Indicators	Log K_{ow} (dimensionless)	Low	Low (no quantitative data)
Benzidine-based Cationic Indicators	Effective minimum cross-sectional diameter (D_{min}, nm)	N/A	N/A
Benzidine-based Cationic Indicators	Effective maximum cross-sectional diameter (D_{max}, nm)	N/A	N/A
Benzidine-based Cationic Indicators	pK_a (dimensionless)	pK_{a1} = not reliable pK_{a2} = not reliable	NA

Abbreviations: K_a, acid dissociation constant; K_{ow}, octanol–water partition coefficient; N/A, not applicable; NA, not available (C.I.generic name)

Data for each of the Benzidine-based Precursors and Benzidine Derivatives are shown individually in Tables 3-2 and 3-3, respectively, since there are important structural differences between these substances. More detailed data for each of these substances are also available in Appendix A, Tables A-7 and A-8. While physical and chemical properties also vary from one substance to another, generally, Benzidine Derivatives are water soluble, with low to moderate log K_{ow} and log organic carbon–water partition coefficient (log K_{oc}) values. The two Benzidine-based Precursors are less water soluble, with higher log K_{ow} and log K_{oc} values.

The only experimental physical and chemical property data available for the two Benzidine-based Precursors were for melting point; therefore, model estimates were used for the remaining properties for the neutral form of the substances (Table 3-2).

It is acceptable to use (Q)SAR models for Benzidine-based Precursors since they are relatively simple structures and fit in the model domains of applicability. Benzidine-based Precursors have high modelled log K_{ow} and log K_{oc} values and relatively low water solubility in comparison with the Benzidine Derivatives. However, there are differences between these two substances, as Naphthol AS-BR is practically water insoluble and does not ionize readily based on pK_a, while TCDB is somewhat soluble and is predicted to ionize in water.

Table 3-2. Experimental and estimated physical and chemical properties (at standard temperature of approximately 25°C) for the Benzidine-based Precursors

Chemical	Property	Value(s) or range (for more than 3 data points)	Pivotal value used in this assessment (basis for selection)
Naphthol AS-BR	Melting point (°C)	246; 350	290 (mean of estimated data)
Naphthol AS-BR	Boiling point (°C)	927.49	927.49 (sole estimated value)
Naphthol AS-BR	Vapour pressure (Pa)	7.7×10^{-25}	7.7×10^{-25} (sole estimated value)
Naphthol AS-BR	Henry's Law constant (Pa·m^3/mol)	1.96×10^{15}	1.96×10^{-15} (sole estimated value)
Naphthol AS-BR	Log K_{ow}	7.75	7.75 (sole estimated value)
Naphthol AS-BR	Log K_{oc}	1.43×10^5; 8.21×10^5	4.85×10^5 (mean of estimated values)
Naphthol AS-BR	Log K_{oa}	25.85	25.85 (sole estimated value)
Naphthol AS-BR	Water solubility (mg/L)	8.97×10^{-6}; 1.44×10^{-5}	1.15×10^{-6} (mean of estimated values)
Naphthol AS-BR	Effective minimum cross-sectional diameter (D_{min}, nm)	1.02	1.02 (sole estimated value)
Naphthol AS-BR	Effective maximum cross-sectional	1.42	1.42 (sole estimated value)

Chemical	Property	Value(s) or range (for more than 3 data points)	Pivotal value used in this assessment (basis for selection)
	diameter (D_{max}, nm)		
Naphthol AS-BR	pK_a (dimensionless)	$pK_{a1} = 13.80$ $pK_{a2} = 13.10$	$pK_{a1} = 13.80$ $pK_{a2} = 13.10$ (sole estimated values)
TCDB	Melting point (°C)	250.21	250.21 (sole estimated value)
TCDB	Boiling point (ºC)	580.51	580.51 (sole estimated value)
TCDB	Vapour pressure (Pa)	3.16×10^{-8}	3.16×10^{-8} (sole estimated value)
TCDB	Henry's Law constant ($Pa \cdot m^3/mol$)	5.81×10^{-9}	5.81×10^{-9} (sole estimated value)
TCDB	Log K_{ow}	5.13	5.13 (sole estimated value)
TCDB	Log K_{oc}	2.2; 5.47	3.85 (mean of estimated values)
TCDB	Log K_{oa}	16.76	16.76 (sole estimated value)
TCDB	Water solubility (mg/L)	0.26; 32.80	16.53 (mean of estimated values)
TCDB	Effective minimum cross-sectional diameter (D_{min}, nm)	0.83	0.83 (sole estimated value)
TCDB	Effective maximum cross-sectional diameter (D_{max}, nm)	1.05	1.05 (sole estimated value)

Chemical	Property	Value(s) or range (for more than 3 data points)	Pivotal value used in this assessment (basis for selection)
TCDB	pK_a (dimensionless)	$pK_{a1} = -3.9$ $pK_{a2} = -5.15$	$pK_{a1} = -3.9$ $pK_{a2} = -5.15$ (sole estimated values)

Abbreviations: K_a, acid dissociation constant; K_{oa}, octanol–air partition coefficient; K_{oc}, organic carbon–water partition coefficient; K_{ow}, octanol–water partition coefficient

The variance in the physical and chemical properties of the five Benzidine Derivatives is generally quite high due to small differences in their chemical structures (e.g., 3,3′-DMB·2HCl is a salt). Three substances, 3,3′-DMB, 3,3′-DMOB and 3,3′-DMB·2HCl, are primary aromatic amines that are not expected to become ionized under relevant physiological and environmental pH conditions, as indicated by their pK_a values (Table 3.3). 3,3′-DMB is structurally identical to benzidine but contains two methyl (CH_3-) substituents in the *ortho* position to the amino functional groups. 3,3′-DMB·2HCl is anticipated to behave as a salt and will produce 3,3′-DMB once released in solution. 3,3′-DMOB contains two methoxy functional groups ($O–CH_3–$) in the *ortho* position to the two amino groups. TODI contains two isocyanate functional groups (–N=C=O) and two methyl groups in the *ortho* position to the isocyanate functional groups, which will hydrolyze readily when in contact with water (see Environmental Persistence section). 4N-TMB is a tertiary amine and lacks the N–H groups that undergo *N*-hydroxylation.

Any modelled values shown in Table 3-3 and Table A-8 in Appendix A are for the neutral forms of the five Benzidine Derivatives.

Using (Q)SAR models for these substances is acceptable, as the Benzidine Derivatives are simpler substances that fit in the model domains of applicability. It is noted that EPIWIN's predictions for salts are uncertain; therefore, predictions generated for 3,3′-DMB·2HCl will not be considered in the assessment.

The modelled water solubilities, log K_{ow} values and Henry's Law constants for 3,3′-DMB, 3,3′-DMOB and 4N-TMB were determined using the EVA option in WATERNT, KOWWIN and HENRYWIN in EPISuite 4.1. The empirical value for the analogue is quantitatively adjusted based on structural fragment differences when the two chemicals are compared. In the present case, water solubility, log K_{ow} value and Henry's Law constant for the analogue substance benzidine were used to generate predictions for 4N-TMB or to correct predictions for 3,3′-DMB and 3,3′-DMOB.

Table 3-3. Experimental and estimated physical and chemical properties (at standard temperature of approximately 25°C) for the Benzidine Derivatives

Chemical	Property	Value(s) or range (for more than 3 data points)	Pivotal value used in this assessment (basis for selection)
3,3′-DMB	Melting point (°C)	128–132 (*n* = 6)	130 (mean of experimental data)
3,3′-DMB	Boiling point (ºC)	200–339 (*n* = 4)	280 (mean of experimental data)
3,3′-DMB	Vapour pressure (Pa)	9.226×10^{-5}; 2.74×10^{-2}	9.226×10^{-5} (sole experimental value)
3,3′-DMB 3,3′-DMB	Henry's Law constant (Pa·m^3/mol)	6.373×10^{-7} – 2.59×10^{-2} (*n* = 4)	2.59×10^{-2} (sole EVA method value)
3,3′-DMB	Log K_{ow}	2.3–3.0 (*n* = 4)	2.4 (mean of experimental and EVA values)
3,3′-DMB	Log K_{oc}	2.17–3.50	2.8 (mean of estimated values)
3,3′-DMB	Log K_{oa}	10.93	10.93 (sole estimated value)
3,3′-DMB	Water solubility (mg/L)	27–1300 (*n* = 5)	51 (mean of MITI data and EVA values)
3,3′-DMB	Effective minimum cross-sectional diameter (D_{min}, nm)	0.79	0.79 (sole estimated value)
3,3′-DMB	Effective maximum cross-sectional diameter (D_{max}, nm)	0.86	0.86 (sole estimated value)
3,3′-DMB	pK_a (dimensionless)	pK_{a1} = 4.6, 4.5, 3.3 pK_{a2} = 3.4–3.5	pK_{a1} = 4.13 pK_{a2} = 3.45 (mean of experimental

Chemical	Property	Value(s) or range (for more than 3 data points)	Pivotal value used in this assessment (basis for selection)
			data)
3,3′-DMB·2HCl	Melting point (°C)	210; 340	275 (mean of experimental data)
3,3′-DMB·2HCl	Boiling point (ºC)	NA	NA
3,3′-DMB·2HCl	Vapour pressure (Pa)	NA	NA
3,3′-DMB·2HCl	Henry's Law constant (Pa·m^3/mol)	NA	NA
3,3′-DMB·2HCl	Log K_{ow}	NA	NA
3,3′-DMB·2HCl	Log K_{oc}	NA	NA
3,3′-DMB·2HCl	Log K_{oa}	NA	NA
3,3′-DMB·2HCl	Water solubility	10 000; 50 000	30 000 (mean of experimental data)
3,3′-DMB·2HCl	Effective minimum cross-sectional diameter (D_{min}, nm)	0.77	0.77 (sole estimated value)
3,3′-DMB·2HCl	Effective maximum cross-sectional diameter (D_{max}, nm)	0.77	0.77 (sole estimated value)
3,3′-DMB·2HCl	pK_a (dimensionless)	NA	NA
3,3′-DMOB	Melting point (ºC)	136; 137	137 (mean of experimental data)
3,3′-DMOB	Boiling point (ºC)	356; 417.2	356 (sole experimental value)
3,3′-DMOB	Vapour pressure (Pa)	$1.66 \times 10^{-5} – 9.45 \times 10^{-4}$	1.66×10^{-5} (sole experimental value)
3,3′-DMOB	Henry's Law constant (Pa·m^3/mol)	$1.83 \times 10^{-8} – 7.45 \times 10^{-5}$	7.45×10^{-5} (EVA method)
3,3′-DMOB	Log K_{ow}	1.5; 2.08	1.7 (mean of experimental and EVA values)
3,3′-	Log K_{oc}	1.99; 2.71	2.4 (mean of estimated

Chemical	Property	Value(s) or range (for more than 3 data points)	Pivotal value used in this assessment (basis for selection)
DMOB			values)
3,3′-DMOB	Log K_{oa}	13.21	13.2 (sole estimated value)
3,3′-DMOB	Water solubility	60; 146.8	103.4 (mean of experimental and EVA values)
3,3′-DMOB	Effective minimum cross-sectional diameter (D_{min}, nm)	0.79	0.79 (sole estimated value)
3,3′-DMOB	Effective maximum cross-sectional diameter (D_{max}, nm)	0.86	0.86 (sole estimated value)
3,3′-DMOB	pK_a (dimensionless)	pK_{a1} = 4.7; 4.2	4.7 (sole experimental value)
TODI	Melting point (°C)	70–116	71 (mean of experimental data)
TODI	Boiling point (ºC)	314–373	353 (mean of experimental data)
TODI	Vapour pressure (Pa)	2.95×10^{-3}	2.95×10^{-3} (sole estimated value)
TODI	Henry's Law constant (Pa·m^3/mol)	NA	NA
TODI	Log K_{ow}	NA	NA
TODI	Log K_{oc}	NA	NA
TODI	Log K_{oa}	10.47	10.5 (sole estimated value)
TODI	Water solubility	NA	NA
TODI	Effective minimum cross-sectional diameter (D_{min}, nm)	0.77	0.77 (sole estimated value)
TODI	Effective maximum cross-sectional diameter (D_{max}, nm)	0.78	0.78 (sole estimated value)
TODI	pK_a (dimensionless)	NA	NA
4N-TMB	Melting point (°C)	108.5–195 (*n* = 5)	194 (mean of experimental data)
4N-TMB	Boiling point (ºC)	353.7	353.7 (sole estimated value)

Chemical	**Property**	**Value(s) or range (for more than 3 data points)**	**Pivotal value used in this assessment (basis for selection)**
4N-TMB	Vapour pressure (Pa)	2.41×10^{-4}; 2.17×10^{-3}	2.17×10^{-3} (sole experimental value)
4N-TMB	Henry's Law constant (Pa·m^3/mol)	1.06×10^{-2}; 4.94×10^{-1}	4.94×10^{-1} (EVA method)
4N-TMB	Log K_{ow}	3.53; 4.11	3.53 (EVA method)
4N-TMB	Log K_{oc}	2.75; 3.07; 3.17	3 (mean of estimated values)
4N-TMB	Log K_{oa}	9.48	9.48 (sole estimated value)
4N-TMB	Water solubility	8.23–33.8 (*n* = 5)	22.6 (mean of 4N-TMB experimental and EVA values)
4N-TMB	Effective minimum cross-sectional diameter (D_{min}, nm)	0.65	0.65 (sole estimated value)
4N-TMB	Effective maximum cross-sectional diameter (D_{max}, nm)	0.65	0.65 (sole estimated value)
4N-TMB	pK_a (dimensionless)	pK_{a1} = 6.14 pK_{a2} = 4.07	pK_{a1} = 6.14 pK_{a2} = 4.07 (sole estimated values)

Abbreviations: EVA, Experimental Value Adjustment; K_a, acid dissociation constant; K_{oa}, octanol–air partition coefficient; K_{oc}, organic carbon–water partition coefficient; K_{ow}, octanol–water partition coefficient; MITI, Ministry of International Trade and Industry (Japan); NA, not available

4. Sources and Uses

4.1 Sources

All Benzidine-based Dyes, Benzidine-based Cationic Indicators, Benzidine-based Precursors and Benzidine Derivatives are anthropogenically produced and not expected to occur naturally in the environment.

In recent years (2005 to present), all substances included in this Screening Assessment have been included in surveys issued pursuant to section 71 of *CEPA 1999*. Nine substances were included in a survey for the 2005 calendar year (Canada 2006b), 2 of these substances were also included in surveys conducted pursuant to section 71 for the 2006 calendar year under the Challenge Initiative (Canada 2006a, 2006b, 2007, 2008, 2009), 5 substances were included in Phase One of the DSL Inventory Update survey (Canada 2009) and 33 substances were included in a survey conducted pursuant to section 71 of CEPA 1999 for the 2010 calendar year that focused on the Aromatic Azo and Benzidine-based Substance Group (Canada 2011). Three substances were included in both surveys conducted for the 2005 and 2010 calendar years.

No substances were reported as being manufactured or imported into Canada above the reporting threshold of the surveys. Direct Blue 14 was however reported as being imported into Canada, in quantities below or equal to the reporting threshold in the DSL Inventory Update survey. Acid Red 97 had declarations of stakeholder interest. This is consistent with the trend by manufacturers to phase out the use of benzidine-based dyes in the mid- to late 1970s and replace them with other dyes due to potential human health concerns (IARC 2010a). Additionally, restrictions on uses of dyes based on benzidine are in place in many jurisdictions, including Europe (Environment Canada and Health Canada 2013). Two European surveys, as well as a Japanese study, indicate that 3,3′-DMB and 3,3′-DMOB are still sometimes detected in some textiles and leather products, some of which were reported to be imported from other countries. Accordingly, these two Benzidine Derivatives could be present in imported products in Canada, as the Canadian textile market is predominantly composed of imported products. Testing of products on the Canadian market, however, did not identify these two derivatives in imported and domestic textile and leather products (Health Canada 2013).

4.1.1 Benzidine-based Acid Dyes

Based on the information submitted in response to industry surveys conducted for the years 2005 and 2006 under *Canada Gazette* notices issued pursuant to section 71 of

CEPA 1999, no manufacture or import activity in Canada above the reporting threshold was reported for any of the 9 Benzidine-based Acid Dyes (Canada 2006a, 2006b, 2008, 2009, 2011b).

Acid Red 97 had a stakeholder interest expressed in the section 71 survey for the reporting year of 2010 (Environment Canada 2012), but manufacture or import of the substance above the reporting threshold was not reported. Acid Red 97 was reported to be used or sold in Canada in quantities less than the reporting threshold of 100 kg (2010 email from Ecological and Toxicological Association of Dyes and Organic Pigments Association to Program Development and Engagement Division, Environment Canada; unreferenced).

4.1.2 Benzidine-based Direct Dyes

Information on Canadian manufacture and import of Direct Blue 14 was collected in Phase One of the DSL Inventory Update in 2009 under *Canada Gazette* notices issued pursuant to section 71 of CEPA 1999 (Canada 2006b, 2008b, 2011b).

Fewer than four companies reported using Direct Blue 14 in quantities between 0 and 100 kg/year. No information was submitted on other Benzidine-based Direct Dyes in recent regulatory surveys issued under CEPA 1999.

Only one Benzidine-based Direct Dye was found on any European use lists: Direct Blue 295 was identified as an LPV chemical in the EU (ESIS ©1995–2012). No information was found on recent use of Direct Blue 14 by the EU.

4.1.3 Benzidine-based Cationic Indicators

No information was submitted on cationic indicators in recent regulatory surveys issued under CEPA 1999. No information is available from Substances in Preparations in Nordic Countries (SPIN), the US *Toxic Substances Control Act* (TSCA) or the European Chemical Substances Information System (ESIS).

4.1.4 Benzidine-based Precursors

No information was submitted on the two Benzidine-based Precursors (Naphthol AS-BR and TCDB) in recent regulatory surveys issued under CEPA 1999. No information is available from SPIN, TSCA or ESIS.

4.1.5 Benzidine Derivatives

No information was submitted on Benzidine Derivatives in recent regulatory surveys issued under CEPA 1999, with the exception of one stakeholder that identified itself as having an interest in one substance (3,3′-DMOB) (Canada 2006b).

TODI, 3,3′-DMB and 3,3′-DMOB are considered LPV chemicals by the EU (ESIS ©1995–2012). TODI is also registered in the European Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) initiative (ECHA 2012).

The national aggregate production volumes for TODI in the United States were 4 540–227 000 kg (i.e., 10 000–500 000 pounds) and 227 000–454 000 kg (i.e., 500 000 – 1 000 000 pounds) in the 2002 and 2006 reporting cycles under the US EPA’s Inventory Update Reporting program (US EPA 2010, 2012b). 3,3′-DMB·2HCl was last reported in 1990 at a quantity of 4540–227 000 kg (i.e., 10 000 – 500 000 pounds) (US EPA 2012b).

4.2 Uses

Most Benzidine-based Substances have common structural features and similar functional uses as colourants, which are used in various sectors, predominantly textile and leather, as well as research and development (Environment Canada and Health Canada 2013).

No manufacture or import activities were reported in response to the section 71 survey for the calendar year 2010 (Environment Canada 2012) or other recent surveys (Environment Canada 2006b, 2008, 2009) above the reporting threshold, however it was reported that Direct Blue 14 was imported for use as a laboratory substance for research and development below or equal to the reporting threshold of 100 kg (Canada 2009; Environment Canada 2009). General uses, globally, reported in publicly available sources for each subgroup are summarized in Table 4-1 (Merck Index 2001; Kirk-Othmer 2010; Sigma-Aldrich Canada 2010; Ullmann’s Encyclopedia 2010; US EPA 2010).

Table 4-1. General uses of Benzidine-based Substances and Benzidine Derivatives

Benzidine-based Acid Dyes	Benzidine-based Direct Dyes	Benzidine-based Cationic Indicators	Benzidine-based Precursors	Benzidine Derivatives

Dyes, pigments and/or colouring agents Electro-optical devices Food contact substances / food packaging Laboratory agents (e.g., stains) Laser and liquid crystal display Leather Paints and coatings Paper Pharmaceuticals Plastic materials Printing inks Textiles	Chemical intermediates Dyes, pigments and/or colouring agents Electro-optical devices Food contact substances / food packaging Laboratory agents Laser and liquid crystal display Leather Paints and coatings Paper Pharmaceuticals Plastic materials Printing inks Textiles	Laboratory agentsCosmetic Regulations	Intermediates in the manufacturing of: ▪ Dyes, pigments and/or colouring agent ▪ Electro-optical devices ▪ Food contact substances / food packaging ▪ Laboratory agents ▪ Laser and liquid crystal display ▪ Paints and coatings ▪ Paper ▪ Pharmaceuticals	Intermediates in the manufacturing of: ▪ Adhesives, elastomers, flexible and rigid foam plastics ▪ Dyes, pigments and/or colouring agents ▪ Printing inks

Benzidine-based Dyes may be regulated as colorants in Canada by various measures, depending on their uses and applications, as explained further below.

None of the substances in this Screening Assessment are on the *List of Permitted Colouring Agents* as permitted food colourants or identified for use in food packaging materials in Canada (2011 emails from the Food Directorate, Health Canada, to the Risk Management Bureau, Health Canada; unreferenced; Health Canada 2012).

None of the substances in this Screening Assessment are listed as permitted drug colourants, listed for use as non-medicinal ingredients in human drugs in the Therapeutic Products Directorate's internal Non-Medicinal Ingredients Database (2011 email from the Therapeutic Products Directorate, Health Canada, to the Risk Management Bureau, Health Canada; unreferenced) nor have they been identified to be present in veterinary drugs (2011 email from the Veterinary Drugs Directorate, Health Canada, to the Risk Management Bureau, Health Canada; unreferenced) or in biologics in Canada (2011 email from the Biologics and Genetic Therapies Directorate, Health Canada, to the Risk Management Bureau, Health Canada; unreferenced). Direct Blue 14 was the only substance identified in the Drug Products Database as an active ingredient in an ophthalmic solution for humans for use in eye surgery (DPD 2012). None of the substances in this Screening Assessment are listed in the Natural Health Products Ingredients Database (NHPID 2008) as substances in natural health products nor identified in the Licensed Natural Health Products Database (LNHPD 2008) to be present in currently licensed natural health products.

None of the substances in this Screening Assessment are present as ingredients in cosmetic products notified to Health Canada under the *Cosmetic Regulations, Food and Drugs Act (R.S.C., 1985, c. F-27)* (2011 emails from the Consumer Product Safety Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada; unreferenced). None of these substances are included on Health Canada's Cosmetic Ingredient Hotlist[3].

No uses of Benzidine-based Substances or Benzidine Derivatives were identified for pest control products registered in Canada (2011 email from the Pest Management Regulatory Agency, Health Canada, to the Risk Management Bureau, Health Canada; unreferenced).

In addition, no uses of Benzidine-based Substances were identified for military applications in Canada (2011 email from the Department of National Defence to the Risk Management Bureau, Health Canada; unreferenced). Two Benzidine Derivatives,

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[3] The List of Prohibited and Restricted Cosmetic Ingredients (more commonly referred to as the Cosmetic Ingredient Hotlist or simply the Hotlist) is an administrative tool that Health Canada uses to communicate to manufacturers and others that certain substances, when present in a cosmetic, may contravene the general prohibition found in section 16 of the Food and Drugs Act or a provision of the Cosmetic Regulations (Health Canada 2011a).

3,3′-DMOB and 3,3′-DMB, have been identified to be used in military applications in Canada for gas detector kits (2011 email from the Department of National Defence to the Risk Management Bureau, Health Canada; unreferenced).

4.2.1 Benzidine-based Acid Dyes

Acid dyes are used primarily in the textile industry for dyeing natural fibres and synthetics (e.g., wool, silk, nylon, polyesters, acrylic and rayon). The sulfonic acid groups react with the cationic amino groups in the fibres (ETAD 1995b). To a lesser extent, these dyes are used in other applications, such as leather, plastics, inks and paints (ETAD 1995b; Øllgaard et al. 1998; CII 2011).

The dominant use of Acid Red 97 was identified for dyeing leather and textiles (CII 2011).

4.2.2 Benzidine-based Direct Dyes

Direct dyes, because of their larger and planar structure, exhibit high affinity for cellulose fibres and are applied directly to cellulose-containing materials in a neutral dye bath (ETAD 1995b; Hunger 2003). They are used for dyeing cotton, rayon and, to a lesser extent, wool, silk and nylon (Environment Canada and Health Canada 2013). In addition to the general uses of Benzidine-based Direct Dyes, a specific therapeutic use of Direct Blue 14 as an active ingredient in an ophthalmic solution used in eye surgery was identified in Canada (DPD 2012), Direct Red 28 in testing hydrochloric acid in gastric contents and for testing amyloidosis (Merck Index 2001), and Direct Blue 15 as a tint, a type of photographic agent, for cinematographic films (HSDB 1983–).

4.2.3 Benzidine-based Cationic Indicators

TDBPD was identified as being used as a laboratory chemical and in scientific research and development (ChemIDplus 1993; Merck Index 2001; SPIN 2010). It is used as a laboratory agent and intermediate in the synthesis of a dark blue pigment (Merck Index 2001).

4.2.4 Benzidine-based Precursors

The substance TCDB is expected to be used not as a dye but as a stabilized diazo compound that can be used in print pastes for textile printing with azoic dyes (Kirk-Othmer 2010). It can also be used as a precursor or intermediate.

Naphthol AS-BR can be used as a precursor and coupling component to make Naphthol AS diazo pigments and for dyeing cloth and printing textiles with azoic dyes (Kirk-Othmer 2010).

4.2.5 Benzidine Derivatives

Benzidine Derivatives are primarily used as intermediates for the synthesis of colourants and chemicals that are later used in the preparation of other materials, such as textiles, foam, elastomers and plastics (HSDB 1983– ; Lide 2002; ChemicalLand21 2010; Kirk-Othmer 2010; US EPA 2010).

4N-TMB has been used as a chemical intermediate in the manufacture of triarylmethane dyes (Kirk-Othmer 2010).

TODI is used in the manufacture of urethane, plastics and plasticizers. More specifically, TODI is used as a polymer intermediate to produce urethane elastomers used to manufacture seals, automobile parts, specialty polyurethanes and polyurethane adhesives for food packaging (Aznar et al. 2011). TODI is also used as a reactant in the plastics pipe, pipe fitting and unlaminated profile shape manufacturing sector in the United States (US EPA 2010).

3,3′-DMB·2HCl was listed as an intermediate in pigment and dyes manufacturing (Merck Index 2001; Sigma-Aldrich Canada 2010) and in the synthesis of biphenyl and diphenyl ethers. It is also used as a reagent for gold and free chlorine in water (Merck Index 2001).

3,3′-DMOB and 3,3′-DMB are both used as chemical intermediates in the manufacture of azo dyes (Lewis 1997; ATSDR 2001; Merck Index 2001). 3,3′-DMOB may also be used in the production of dyes for dyeing leather, paper, plastic, rubber and textiles (IARC 1974a) and as a chemical intermediate in the production of o-dianisidine diisocyanate (IARC 1974a). 3,3′-DMB may be used as a reagent for gold and free chlorine in water (Merck Index 2001) and as a curing agent for urethane resins (Lewis 1997).

None of the Benzidine Derivatives were identified for use in food packaging materials in Canada (2011 emails from the Food Directorate, Health Canada, to the Risk Management Bureau, Health Canada; unreferenced). Although TODI was identified as a diisocyanate used in polyurethane adhesives in food packaging laminates in Europe (Aznar et al. 2011), there are no indications of this use in Canada (2012 email from the Food Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada; unreferenced).

5. Environmental Fate and Behaviour

The environmental fate of chemicals describes the processes by which chemicals move 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), the Equilibrium Criterion model or EQC (2003) is not applicable for strongly ionic acid and direct dyes and charged, basic substances such as cationic indicators, as they do not fall under the model domain. Therefore, the environmental fate and compartmentalization of these substances will be discussed qualitatively using information on physical and chemical properties. EQC will be used to help characterize and quantify the environmental behaviour of Benzidine Derivatives and Benzidine-based Precursors.

5.1 Releases to Water and Sediment

Dyes (including Benzidine-based Acid and Direct Dyes as well as Benzidine-based Cationic Indicators) have an inherently high affinity to substrates, which results in high fixation rates (ETAD 1995b; Environment Canada and Health Canada 2013). If released to natural waters or wastewater in an untransformed state, negatively charged Benzidine-based Acid and Direct Dyes are expected to primarily bind to suspended organic matter, specifically positively charged particulates, due to electrostatic interactions and eventually settle out to bed sediments or wastewater sludge (ETAD 1995b). Positively charged Benzidine-based Cationic Indicators are expected to have an affinity for ionic substrates such as dissolved organic material, which has a net negative charge due to the presence of humic and fulvic acids. 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). After partitioning to sediment or wastewater sludge, some azo dyes may bind reversibly and become resuspended, while others will bind irreversibly and remain buried.

A smaller proportion of ionic dyes may also reside in the water column due to their very high water solubility. Eventually, these dyes may form associations with organic material and settle out to sediments.

Certain dyes based on benzidine may also be biotransformed in sediment to benzidine derivatives. EQC analysis was performed for 3,3′-DMB, 3,3′-DMOB and 4N-TMB, which are relatively neutral substances except at lower pHs. Since 3,3′-DMB·2HCl is a salt of 3,3′-DMB and TODI hydrolyzes to 3,3′-DMB, these three substances are expected to behave similarly to the other two Benzidine Derivatives. If Benzidine Derivatives are completely released to water (at a level of 100%), EQC modelling indicates that more than 90% of the mass of Benzidine Derivatives will remain in water, with only a small percentage (0.2–9.3%) going to sediment. However, as indicated in Environment Canada and Health Canada (2013), sorption is an important fate process for aromatic amines in aquatic and sediment systems (Colon et al. 2002; Chen and Nyman 2009); therefore, it is anticipated that a greater percentage of Benzidine Derivatives will be found in sediment than is predicted by EQC.

When Benzidine-based Precursors are released to water only, EQC modelling indicates that 99.1% of TCDB will remain in water, with less than 1% going to sediment due to its moderate solubility. This contrasts with a prediction of 92.9% of Naphthol AS-BR going to sediment, with only 7.1% staying in water, due to its very low solubility.

5.2 Releases to Soil

There are two major routes for the release of azo dyes to soil: directly via the use or application of a dye in the environment and indirectly via the application of wastewater biosludge 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). Specifically, acid dyes have an inherently high affinity for substrates, with fixation levels ranging from 85% to 98% for acid dyes with more than one sulfonic acid group (ETAD 1995b).

Certain azo dyes may also biotransform in soil to Benzidine Derivatives. If Benzidine Derivatives are completely released to soil, EQC modelling for 3,3′-DMB, 3,3′-DMOB and 4N-TMB indicates that the majority (81–99%) of them are expected to remain in soil . Again, 3,3′-DMB·2HCl and TODI are expected to behave similarly.

Using EQC modelling, the two Benzidine-based Precursors, Naphthol AS-BR and TCDB, are both expected to remain in soil when released to that medium (99.9% and

96.6%, respectively), with only a small amount going to air in the case of TCDB (3.3%) and negligible amounts going to sediment (< 0.1% for both substances).

5.3 Releases to Air

Benzidine-based Dyes are not expected to be released to air and are not expected to partition to this compartment due to very low vapour pressures and Henry's Law constants (HSDB 1983– ; Øllgaard et al. 1998). Water-soluble dyes such as Benzidine-based Acid and Direct Dyes as well as Benzidine-based Cationic Indicators 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 1995b; Øllgaard et al. 1998).

Benzidine Derivatives and Benzidine-based Precursors are also not expected to be released to air. However, if released entirely to air, the majority of Benzidine Derivatives and Benzidine-based Precursors are not expected to stay in this medium, but mostly are predicted by EQC modelling to be deposited to soil (78.6–97.5%).

Given low levels of volatility and physicochemical preference for partitioning to other media, it is also not expected that water-soluble or water-insoluble azo dyes will be subject to long-range atmospheric transport.

5.4 Environmental Persistence

In order to characterize the environmental persistence of Benzidine-based Dyes, Benzidine-based Cationic Indicators, Benzidine-based Precursors and Benzidine Derivatives, empirical and modelled data for these substances were considered under both aerobic and anaerobic conditions. In addition, the process of ecological biotransformation was explored, specifically with respect to the potential for Benzidine-based Dyes to degrade to benzidine derivatives.

5.4.1 Empirical Data for Persistence

Empirical biodegradation data related to the persistence of Benzidine-based Dyes and Benzidine-based Precursors are limited. Select empirical tests (Table 5-1) under aerobic conditions, using standard Organisation for Economic Co-operation and Development (OECD Test Guideline 301C) methodologies, showed no degradation after 42 and 28 days for Acid Red 114 and Direct Black 38, respectively (CHRIP ©2002-2012).

Some conflicting degradation data for azo and benzidine-based dyes have been found. For example, Idaka et al. (1985) reported relatively high colour loss for the benzidine dye Direct Red 28 at 85% and the azo dye Acid Orange 7 at 65% after 150 days. However, since sludge was acclimated to the dyes, longer test periods were used and the percent colour loss was provided instead of percent theoretical oxygen demand uptake, it makes the values difficult to compare with the results of standard OECD Test Guideline 301C tests. Also, it is unclear whether commercial formulations may have been used in some of these tests instead of high-purity dyes.

Table 5-1. Select empirical data for biodegradation of Benzidine-based Dyes under aerobic conditions

Subgroup (specific substance with data)	Method	Degradation value (%)[a]	Degradation endpoint	Test duration (days)	Reference
Benzidine-based Acid Dyes (Acid Red 114)	OECD Test Guideline 301C	0	Biodegradation	42	CHRIP ©2002-2012
Benzidine-based Direct Dyes (Direct Black 38)	OECD Test Guideline 301C	0	Biodegradation	28	CHRIP ©2002-2012

[a] Percent biodegradation at a given concentration of the test substance.

In addition to biodegradation tests under aerobic conditions, it is notable that data for Direct Blue 14 showed 50% degradation in anaerobic sediment after 16 days (Table 5-2).

Table 5-2. Select empirical data for biodegradation of Benzidine-based Dyes under anaerobic conditions

Subgroup (specific substance with data)	Method	Degradation value (%)[a]	Degradation endpoint	Test duration (days)	Reference
Benzidine-based Direct Dyes (Direct Blue 14)	Not listed	50	Water/sediment biodegradation	16	Weber 1991

[a] Percent biodegradation at a given concentration of the test substance.

Significantly more empirical data were available on the Benzidine Derivatives. An empirical abiotic degradation study (hydrolysis) conducted according to OECD Test Guideline 111 (hydrolysis as a function of pH) and EU Method C.7 is available for TODI (ECHA 2012). Results of the study conducted at three pHs (pH 4, 7 and 9) and two temperatures (25°C and 50°C) show rapid hydrolysis rates for TODI, with half-lives of 16 hours (at 25°C and pH 7), 1.2 hours (at 50°C and pH 7) and equal to or less than 2 minutes under all other experimental conditions. The main hydrolysis product identified was 3,3′-DMB, but a second unidentified compound with a molecular weight of 240 g/mol was also observed (ECHA 2012).

Empirical biodegradation studies have been identified for 3,3′-DMB, 3,3′-DMOB and TODI (Table 5-3).

Results from these studies vary for 3,3′-DMB and 3,3′-DMOB. Indeed, 28-day ready biodegradability (Japanese) Ministry of International Trade and Industry (MITI) tests conducted with an initial substance concentration of 100 mg/L indicate that 3,3′-DMB and 3,3′-DMOB are not readily biodegradable (Kawasaki 1980; MITI 1992). Another study conducted by Baird et al. (1977) using conventional Warburg bioassay techniques with 3,3′-DMB and 3,3′-DMOB at a concentration of 20 mg/L at 25°C in activated sludge determined that both substances were completely degraded (100%) over 6 hours. It is noted that the activated sludge samples were chosen from two treatment facilities that combined domestic and industrial discharges; therefore, microorganisms may have been acclimated to aromatic amines. Also, the substances were degraded in spite of some inhibitory effects on the oxygen uptake of microorganisms, indicating that the effects may have been caused by the unidentified degradation products (Baird et al. 1977).

Biodegradation of 3,3′-DMOB was investigated using a 28-day OECD Test Guideline 301A test at six laboratories (Brown and Laboureur 1983). The tests were carried out using the substance at concentrations of 20 and 30 mg/L of sludge, predominantly from domestic discharges. The OECD Test Guideline 301A method was modified, and varying quantities of yeast extracts were added. Complete degradation (100%) was obtained at a high yeast concentration; however, a clear dependence of the extent of degradation on high levels of yeast was observed, indicating that the substance is “not readily biodegradable” (Brown and Laboureur 1983).

The aerobic and anaerobic biodegradabilities of 3,3′-DMOB were investigated by Brown and Hamburger (1987). 3,3′-DMOB was more than 75% degraded in a 28-day ready biodegradability screening test (OECD Test Guideline 301E) with 30 mg/L of innoculum sludge under aerobic conditions. 3,3′-DMOB was completely degraded after 42 days under anaerobic conditions using a protocol identical to the one used in Brown and Laboureur (1983).

Results from a 28-day ready biodegradability closed bottle test (OECD Test Guideline 301D) on TODI (ECHA 2012) showed 0% degradation, indicating that the substance is not readily biodegradable. Since TODI is known to rapidly hydrolyse to 3,3′-DMB in water, this result may also confirm the recalcitrant nature of 3,3′-DMB.

Table 5-3. Empirical data for degradation of Benzidine Derivatives

Chemical	Medium	Fate process	Degradation value	Degradation endpoint (test duration)	Reference
3,3′-DMB	Water	Biodegradation	6% (HPLC) 3% (BOD) (100 mg/L)	% biodegradation (28 days)	MITI 1992
3,3′-DMB	Waste water	Biodegradation	100% (20 mg/L)	% biodegradation (6 h)	Baird et al. 1977
3,3′-DMOB	Water	Biodegradation	Resistant to biodegradation	% biodegradation (28 days)	Kawasaki 1980 MITI 1992
3,3′-DMOB	Waste water	Biodegradation	100% (20 mg/L)	% biodegradation (6 h)	Baird et al. 1977
3,3′-DMOB	Waste water	Biodegradation	0–100%	% biodegradation (28 days)	Brown and Laboureur 1983
3,3′-DMOB	Water	Biodegradation	> 75%	% biodegradation (28 days)	Brown and Hamburger 1987
3,3′-DMOB	Water	Biodegradation	100%	% biodegradation (anaerobic) (42 days)	Brown and Hamburger 1987
TODI	Water	Hydrolysis	100% in 29 h $t_{½}$ = 16 h	% hydrolysis (25°C)	ECHA 2012
TODI	Water	Biodegradation	−4.8%	% biodegradation (28 days)	ECHA 2012

Abbreviations: BOD, biological oxygen demand; HPLC, high-performance liquid chromatography; $t_{½}$, half-life

5.4.2 Modelling of Persistence

In addition to the experimental data, a (Q)SAR-based weight of evidence approach (Environment Canada 2007) 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 Benzidine-based Dyes are expected to be released to this compartment, aerobic biodegradation in water was primarily examined.

Tables A-9a–A-9e in Appendix A summarize the results of available (Q)SAR models for degradation in various environmental media. Aquatic degradation models used in this analysis were HYDROWIN (2010), BIOWIN Submodels 3–6 (BIOWIN 20010, DS TOPKAT (©2005–2009) and CATALOGIC (©2004–2011).

All of the model outputs for Benzidine-based Dyes and Benzidine-based Cationic Indicators as well as all but one model output for Benzidine-based Precursors (BIOWIN Submodel 4) consistently predicted that these substances would biodegrade slowly in water under aerobic conditions (Appendix A, Tables A9a–d). 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.

Table A-9e in Appendix A summarizes the results of available (Q)SAR models for degradation of Benzidine Derivatives in various environmental compartments. Biodegradation in water was primarily examined for substances in the Benzidine Derivatives subgroup, given the ecological importance of the water compartment and the fact that they are expected to be released to this compartment. Only TODI contains a functional group (isocyanate) expected to undergo hydrolysis. Atmospheric degradation predictions were also generated, since Benzidine Derivatives can be semi-volatile.

Gaseous amines have the potential to undergo atmospheric oxidation reactions with hydroxyl, nitrogen oxide or ozone radicals (Ge et al. 2011). Modelled and calculated half-lives of 0.052–0.25 day for the Benzidine Derivatives subgroup indicate that these substances rapidly photooxidize via reaction with hydroxyl radicals (Meylan and Howard 1993; AOPWIN 2010). Degradation of the Benzidine Derivatives via hydroxyl radicals will decrease if the substances are bound to particulate matter. Removal of amines by reactions with ozone is generally negligible (Ge et al. 2011), and no predictions could be made for the Benzidine Derivatives subgroup using AOPWIN (2010).

Overall, all model results generated for 3,3′-DMB, 3,3′-DMOB and 4N-TMB indicate that while some transformation of the parent structure may occur (primary degradation) for 3,3′-DMOB, all three substances are not expected to significantly biodegrade in the environment.

5.4.3 Aerobic Biodegradation of Benzidine-based Dyes and Benzidine-based Cationic Indicators

The available empirical and modelled persistence data for Benzidine-based Acid and Direct Dyes as well as Benzidine-based Cationic Indicators in aerobic environments are in agreement and show little to no biodegradation in the time scale of the studies. This is consistent with the understanding that dyes must be stable to end use applications in order to be effective and that most are generally considered non-degradable under environmentally relevant aerobic conditions (Pagga and Brown 1986; Øllgaard et al. 1998; ETAD 1995b).

5.4.4 Anaerobic Biodegradation of Benzidine-based Dyes and Benzidine-based Cationic Indicators

Under anaerobic or reducing conditions, biotic degradation of dyes may take place relatively rapidly (Yen et al. 1991; Baughman and Weber 1994; ETAD 1995b; Øllgaard et al. 1998; Isik and Sponza 2004). Dyes have a high tendency to cleave at the azo bond, with the formation of aromatic amines (Øllgaard et al. 1998; Hunger 2005). Direct Blue 14 has been documented to degrade in anaerobic sediment–water systems with a half-life of 16 days (Weber 1991). Total mineralization or further degradation of these metabolites could take place if they are transferred (e.g., by sediment resuspension) to aerobic environments (Øllgaard et al. 1998; Isik and Sponza 2004).

In order to account for potentially harmful transformation products, the scientific literature was consulted, and the (Q)SAR-based predictive tool CATALOGIC (©2004–2011) was used to determine what benzidine derivatives may be released from Benzidine-based Dyes and Benzidine-based Cationic Indicators in the environment. Simulations were generated using CATALOGIC’s metabolite simulator based on biological oxygen demand (BOD) data from MITI. Potential degradation products were prioritized based on the percent likelihood of formation, potential hazard and the known commercial activity of their parent compounds. Experimental evidence from Brown and Hamburger (1987) indicates that Direct Blue 14 and Acid Red 114 (a close analogue to Acid Red 111) have the potential to transform to 3,3′-DMB in sediment under anaerobic conditions. Based on the structural similarities between Acid Red 114 and Acid Red 111, it is anticipated that Acid Red 111 would also transform to 3,3′-DMB under similar conditions. Empirical degradation studies conducted with Direct Black 38 identified three different metabolites—namely, aniline (CAS RN 62-53-3) (Isik and Sponza 2004), benzidine and 4-biphenylamine (CAS RN 91-67-1) (Isik and Sponza 2004; Bafana et al.

2009a). However, none of these metabolites are included in the Benzidine Derivatives subgroup assessed in this report.

Modelled data from CATALOGIC are consistent with these empirical results. 3,3′-DMB was identified as a potential degradation product for Direct Blue 14, Acid Red 111 and Acid Red 114, but not for Direct Black 38. Therefore, both Direct Blue 14 and Acid Red 114 have the potential to transform to 3,3′-DMB, one of the Benzidine Derivatives being assessed in this report, under conditions that favour biodegradation of the compounds. The contribution of these transformation products will be discussed further in the Ecological Exposure Assessment section.

Anaerobic biodegradation of the Benzidine-based Cationic Indicators is not expected, as no azo bond is contained within their chemical structures.

5.4.5 Hydrolysis of Benzidine-based Dyes, Benzidine-based Cationic Indicators and Benzidine-based Precursors

The majority of the Benzidine-based Acid and Direct Dyes, Benzidine-based Cationic Indicators and Benzidine-based Precursors 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, four substances, Acid Orange 56 and BADB (Benzidine-based Acid Dyes), BDAAH (a Benzidine-based Direct Dye) and Naphthol AS-BR (a Benzidine-based Precursor), contain amide functional groups that were flagged by EPISuite as having the potential to undergo some degree of hydrolysis.

5.4.6 Summary of Persistence

Due to the persistence of Benzidine-based Dyes, Benzidine-based Cationic Indicators, Benzidine-based Precursors and Benzidine Derivatives subgroups 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 Benzidine-based Dyes, Benzidine-based Cationic Indicators, Benzidine-based Precursors and Benzidine Derivatives. Experimental data for traditional bioaccumulation metrics such as bioconcentration factor (BCF) are minimal and restricted to the water compartment for these substances. In addition, the use of (Q)SAR bioaccumulation modelling was not pursued for Benzidine-based Dyes and Benzidine-based Cationic Indicators, since these substances were outside the model domains of applicability.

5.5.1 Octanol–Water Partition Coefficient

As indicated in Table 4a, Benzidine-based Dyes and Benzidine-based Cationic Indicators have relatively high water solubilities (> 500–116 000 mg/L), and a limited number of experimental data for dyes suggest relatively low log K_{ow} values (below 0.77), which would also suggest a very low bioaccumulation potential according to equilibrium partitioning theory. This is consistent with the general view from other sources that note the very low bioaccumulation potential of ionic dyes (ETAD 1995b).

Benzidine-based Precursors have high modelled log K_{ow} values. Naphthol AS-BR has a modelled log K_{ow} of 7.75, which, at above 7, is just over the threshold of indicating reduced bioaccumulation potential (Arnot and Gobas 2003). TCDB has a modelled log K_{ow} of 5.13, which may be indicative of a high potential for bioaccumulation.

Benzidine-based Derivatives are moderately to highly water soluble (8.23–50 000 mg/L), and most have relatively low log K_{ow} values (below 3.0). Modelled log K_{ow} values for 4N-TMB ranged from 3.53 to 4.11, which suggests a moderate bioaccumulation potential. Estimated log K_{oa} values ranging from 9.48 to 13.21 suggest that, given a terrestrial dietary exposure, substances in the Benzidine-based Derivatives subgroup would not have the potential to biomagnify in terrestrial food webs, as suggested by Gobas et al. (2003) and Kelly et al. (2007), assuming some degree of metabolism (at least a metabolic rate constant of ≥ 0.03/day) and some degree of assimilation efficiency from the diet.

5.5.2 Bioconcentration Factor (BCF)

Estimated and experimental log K_{ow} values were compared with experimental BCFs for fish for a number of dyes (Anliker et al. 1981; ETAD 1995b; Øllgaard et al. 1998). With respect to the data for six acid dyes and one direct dye, reported BCFs were less than 10, indicating that these very hydrophilic (ionic) dyes are not likely to bioconcentrate in aquatic organisms. Data available for Acid Red 114 (Table 5-4) illustrate low BCF values (42–84 L/kg) for carp exposed to two different concentrations.

Table 5-4. Empirical data for bioconcentration of Benzidine-based Dyes (Acid Red 114)

Test organism	Experimental concentration (mg/L)	Endpoint (BCF, L/kg)	Reference
Common carp (*Cyprinus carpio*)	0.2	42–76	MITI 1992
Common carp (*Cyprinus carpio*)	0.02	52–84	MITI 1992

No experimental BCF data were available for the Benzidine-based Precursors, but estimated data are presented in Table 5-5, as (Q)SAR models were deemed acceptable for these substances based on their simpler chemical structures.

Modelled data for Naphthol AS-BR show some potential for bioaccumulation, with values above 5000, without factoring in metabolism. However, when metabolism is taken into consideration, the BCF value lowers significantly. TCDB shows moderate to high BCF values (1120–6456) before metabolism is factored in, but values are of much lower concern when metabolism is considered. However, since the log K_{ow} and all of the BCF data are modelled, there is a high degree of uncertainty regarding the bioaccumulation potential of Naphthol AS-BR and TCDB and reason for some concern.

Table 5-5. Modelled BCF data for Benzidine-based Precursors

Substance	Log K_{ow}	Metabolism constant (k_M) (/day)	Test organism	Model and model basis	Value wet weight (L/kg)	Reference
Naphthol AS-BR	7.75	NA	Fish	BCFBAF Submodel 1 (linear regression)	5699	BCFBAF 2010
Naphthol AS-BR	7.75	0.2909 (10 g fish)	Fish	BCFBAF (with biotransformation rate; mid-trophic)	106.2	BCFBAF 2010
Naphthol AS-BR	7.75	0.304	Fish	BCF_{max}	12 302 (Log	CPOPs 2008

Substance	**Log K_{ow}**	**Metabolism constant (k_M) (/day)**	**Test organism**	**Model and model basis**	**Value wet weight (L/kg)**	**Reference**
					BCF_{max}: 4.09)	
Naphthol AS-BR	7.75	0.304	Fish	BCF corrected	7.4 (Log BCF corrected: 0.871)	CPOPs 2008
TCDB	5.13	NA	Fish	BCFBAF Submodel 1 (linear regression)	1120	BCFBAF 2010
TCDB	5.13	0.192 (10 g fish)	Fish	BCFBAF (with biotransformation rate; mid-trophic)	1623	BCFBAF 2010
TCDB	5.13	0.00363	Fish	BCF_{max}	6456 (Log BCF_{max}: 3.81)	CPOPs 2008
TCDB	5.13	0.00363	Fish	BCF corrected	645.7 (Log BCF corrected: 2.81)	CPOPs 2008

While experimental data were also limited, BCF values were available for one of the five Benzidine Derivatives (3,3′-DMB). Low BCFs ranging from 4.8 to 83 in fish over a period of 56 days indicate that 3,3′-DMB has a low bioaccumulation potential (Table 5-6).

Low BCFs (55–2617) have also been observed for benzidine in fish, *Daphnia magna*, mosquito larvae, snails and filamentous green algae (Lu et al. 1977; Freitag et al. 1985;

Tsuda et al. 1997). Based on these values, it is anticipated that the substances from the Benzidine Derivatives subgroup have a low bioaccumulation potential.

Table 5-6. Empirical data for bioconcentration of Benzidine Derivatives (3,3′-DMB)

Test organism	Experimental concentration (mg/L)	Endpoint (BCF, L/kg)	Reference
Fish	Not known	47	SRC 2011
Common carp(*Cyprinus carpio*)	0.2	4.8–34	MITI 1992
Common carp (*Cyprinus carpio*)	0.02	10–83	MITI 1992

Modelled bioaccumulation factor (BAF)/BCF data for Benzidine Derivatives are in general agreement with experimental values, showing low bioaccumulation potential in the range of 5.2–1173.

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 Benzidine-based Dyes and Benzidine-based Cationic Indicators, available data on water solubility, molecular weight and cross-sectional diameter are considered in order to determine 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 high water solubility of these substances.

In general, Benzidine-based Dyes are relatively hydrophilic, large molecules with high molecular weight (696–1134 g/mol). The minimum and maximum cross-sectional diameters for Benzidine-based Dyes range from 0.95 nm (D_{min}) to 2.07 nm (D_{max}) (Table 4a). These characteristics suggest a low bioaccumulation potential for these substances.

Cross-sectional diameters for the Benzidine-based Cationic Indicators could not be determined by the models.

Effective diameters of the Benzidine Precursors ranged from 0.83 nm (D_{min} for TCDB) to 1.42 nm (D_{max} for Naphthol AS-BR) (Table 3-2). This indicates that Naphthol AS-BR may have somewhat less bioaccumulation potential than TCDB.

Benzidine Derivatives were found to have effective cross-sectional diameters that ranged from 0.65 nm (D_{min}) to 0.86 nm (D_{max}) (Table 3-3). Benzidine Derivatives evaluated in this assessment generally have low molecular weights (212–285 g/mol) and low effective cross-sectional diameters. Therefore, this would not be a factor in restricting the rate of uptake when crossing cell membranes.

5.5.4 Summary of Bioaccumulation Potential

Benzidine-based Dyes and Benzidine-based Cationic Indicators are expected to have a low bioaccumulation potential due to low observed bioconcentration in empirical tests. This is supported by and consistent with their physical and chemical properties (i.e., low log K_{ow}, ionized at relevant environmental pH, high molecular weight, large cross-sectional diameters, high water solubility) and likely high degree of biotransformation by organisms. Benzidine-based Precursors are expected to be moderately bioaccumulative due to their high modelled BCF values without consideration of metabolism and physical and chemical properties (i.e., moderate to high log K_{ow}, molecular weight and cross-sectional diameters that do not slow uptake rates). Low experimental and modelled BCF and BAF data for Benzidine Derivatives, physical and chemical property information (i.e., high water solubility, low log K_{ow}) that suggests a low lipid partitioning tendency plus an inherent ability to be biotransformed in organisms indicate consistently that these substances have low bioaccumulation potential.

6. Potential to Cause Ecological Harm

6.1 Ecological Effects Assessment

Only empirical data (from specific substances within the subgroups and analogues) were considered for assessment of the ecological effects potential of Benzidine-based Dyes given the high level of uncertainty associated with modelling the ecotoxicity of these substances. With respect to the Benzidine Derivatives, empirical and modelled data were considered.

6.1.1 Empirical Studies for the Aquatic Compartment

A variety of lines of evidence have been used to determine the ecotoxicological potential of the Benzidine-based Dyes, Benzidine-based Precursors and Benzidine Derivatives.

Limited empirical studies were available for Benzidine-based Dyes (Table 6-1). Most substances had very few or no empirical data, whereas other substances, such as Direct Blue 14, had multiple studies in the aquatic compartment.

One empirical toxicity study was available in which the acute toxicity of various dyes (46 in total) to fathead minnow (*Pimephales promelas*) was studied (Little and Lamb 1972). Bioassays were carried out according to published standard methods, and data sheets for each test were prepared, including pertinent information on the test organisms, dilution water and test conditions. The experiment was designed to estimate the threshold concentration at which 50% of the experimental animals survived after 96 hours (TL_{50}). The TL_{50} for 29 dyes, including 2 dyes from the Benzidine-based Direct Dyes subgroup (Direct Brown 95 and Direct Black 38), was in excess of 180 mg/L (highest concentration tested).

Empirical toxicity studies for Benzidine-based Acid Dyes were available for Acid Red 114 (MITI 1992) and a formulated product, Lanasyn Scarlet F-3GL 130, which contains Acid Red 111 (Study Submission 2007). Since the Acid Red 111 study, which yielded a 48-hour median lethal concentration (LC_{50}) of 11 mg/L for rainbow trout (*Oncorhynchus mykiss*), used a formulation with an unknown concentration of the Benzidine-based Acid Dye and additional study details were not reported, it was not considered to be used as a critical value but is considered for comparative purposes in the weight of evidence. The study on Acid Red 114 by MITI (1992) was done on common carp (*Cyprinus carpio*) and yielded the lowest value for Benzidine-based Acid Dyes, with an LC_{50} of 4 mg/L (Table 9a). These two studies suggest that Acid Red 111 and Acid Red 114 would have a moderate (bordering on high) potential for toxicity.

Toxicity studies on the freshwater shrimp (*Desmocaris trispinosa*) were available for two Benzidine-based Direct Dyes (Direct Blue 14 and Direct Red 2). The 96-hour LC_{50} for Direct Red 2 was reported as 4.96 mg/L, while that for Direct Blue 14 was 11.33 mg/L (Ogugbue and Oranusi 2006). The authors stated that the difference in the toxicities may be partially explained by the lower molecular weight of Direct Red 2 compared with Direct Blue 14 (i.e., lower molecular weight substances are transported more rapidly across the cell membrane). This pattern appeared more evident when a lower molecular mass substance (Mordant Black 17, CAS RN 2538-85-4) and two higher molecular weight substances (Reactive Red 4, CAS RN 17681-50-4and Reactive Yellow 2, CAS RN 50662-99-2) were included in the data set.

In general, when data from multiple taxa were available, fish species seemed to have a higher tolerance to Benzidine-based Dyes than other taxa (see Table 6-1). Invertebrate and algal species appeared to be more susceptible to Benzidine-based Dyes.

The above results are similar to what has been reported generally for acid and direct dyes in the literature (Øllgaard et al. 1998). The Danish Environmental Protection Agency (EPA) study summarized short-term test results for zebrafish, *Daphnia magna*, algae and bacteria from a study by the Ecological and Toxicological Association of Dyes and Organic Pigment Manufacturers (ETAD) covering 47 dyes of different chemical dye classes (although specific dyes included in the study were not reported). In 96-hour LC_{50} tests on zebrafish, toxicity was observed for two acid dyes between 1 and 10 mg/L, for three acid dyes between 10 and 100 mg/L and for six other acid dyes above 100 mg/L. For direct dyes, all seven dyes tested reported toxicity to zebrafish at levels above 100 mg/L. Effects were observed between 10 and 100 mg/L and above 100 mg/L in 48-hour median effective concentration (EC_{50}) tests (endpoint not specified) on *D. magna* for nine acid dyes. Similar tests with direct dyes resulted in adverse effects on *D. magna* at levels above 100 mg/L. Algal toxicity (measured in 72-hour EC_{50} tests) was observed in two cases for acid dyes below 1 mg/L, as well as above this level (for the remaining seven acid dyes), whereas toxicity results for the seven direct dyes tested were all above 1 mg/L. Algae appeared to be the most susceptible organisms for all dye classes tested; the effect was thought to be related to light inhibition at high dye concentrations (coloration of water can occur above 1 mg/L). Generally, bacteria were the least susceptible to most of the different classes of dyes tested, compared with the other organisms (median inhibitory concentrations [IC_{50}s] > 100 mg/L).

Experience with over 200 acid dyes has led to the observation that the potential ecotoxicity of such substances may generally be predicted by the number of sulfonic acid groups present (US EPA 2002). Some monosulfonated and disulfonated dyes have shown high to moderate toxicity (i.e., acute values < 1 mg/L and < 100 mg/L, respectively) to fish and other aquatic organisms. Dyes with three or more acid groups showed low toxicity (i.e., acute values > 100 mg/L) towards fish and invertebrates. All acid dyes showed moderate toxicity to green algae, with further analysis suggesting that such effects may have been related to light shading. For these generalizations to be applicable, the acid dyes must have some water solubility, and molecular weights generally need to be near or below 1 000, which is the case for Acid Red 111.

Furthermore, Environment Canada has evaluated numerous acid dyes under the *New Substances Notification Regulations* and has generally found anionic dyes to be of low toxicity regardless of the number of sulfonic acid groups, but some exceptions have been found (e.g., when a reactive functional group is not hindered).

Few experimental toxicity tests are available for cationic indicators as well. Since cationic indicators are similar in physical and chemical properties to basic (cationic) dyes, some data available for these substances will be used as read-across. In general, cationic indicators are known to have high levels of toxicity that exceed those of acid and direct dyes (Øllgaard et al. 1998). While most available data originate from studies on triarylmethane basic dyes, these are quite different in chemical structure from azo- and benzidine-based dyes. Therefore, for comparative purposes, only benzidine-based basic dye and azo-based basic dye data (for BB4), as well as data for a tetrazolium substructure (TTC), are used in this report for read-across to cationic indicators, as these are structurally more similar substances.

Read-across toxicity data used for Benzidine-based Cationic Indicators range from 2.5 to 10 mg/L, with the most sensitive values being for algae. As noted for acid dyes, the low endpoint value for algae may be due to shading, so, while sensitive, this organism is not the most indicative of cationic indicator toxicity. Most data for Benzidine-based Cationic Indicators are quite old, with studies published in the 1950s, 1960s and 1970s. Fish toxicity data occupy a relatively tight range from 5 to 10 mg/L, with the most reliable, recent and among the most sensitive studies coming from Little and Lamb (1973, 1974), at 5.6 and > 5.6 mg/L for fathead minnow (*Pimephales promelas*) exposed to BB4 for 96 hours.

Table 6-1. Empirical data for aquatic toxicity from representative substances for the Benzidine-based Acid Dyes, Benzidine-based Direct Dyes and Benzidine-based Cationic Indicators subgroups

Subgroup	**Test organism**	**Type of test (duration)**	**Endpoint**	**Value (mg/L)**	**Reference**
Benzidine-based Acid Dyes	Fish (*Cyprinus carpio*)	Acute (96 h)	LC_{50}	4[a] (Acid Red 114)	MITI 1992
Benzidine-based Acid Dyes	Fish (*Oncorhynchus mykiss*)	Acute (48 h)	LC_{50}	11 (Acid Red 111)	Study Submission 2007

Benzidine-based Direct Dyes	Alga (*Scenedesmus subspicatus*)	Chronic (72 h)	EC_{50} (biomass)	4.7 (Direct Blue 15)	Brown 1992
Benzidine-based Direct Dyes	Freshwater shrimp (*Desmocaris trispinosa*)	Acute (96 h)	LC_{50}	4.96 (Direct Red 2) 11.3[a] (Direct Blue 14)	Ogugbue and Oranusi 2006
Benzidine-based Direct Dyes	Fish (*Oncorhynchus mykiss*)	Acute (96 h)	LC_{50}	560 (Direct Blue 15)	Douglas et al. 1986
Benzidine-based Direct Dyes	Fish (*Oryzias latipes*)	Acute (96 h)	LC_{50}	> 1000 (Direct Blue 14)	Tsuji et al. 1986
Benzidine-based Direct Dyes	Fish (*Pimephales promelas*)	Acute (96 h)	LC_{50}	> 180 (Direct Black 38 and Direct Brown 95)	Little and Lamb 1973
Benzidine-based Direct Dyes	Fish (*Danio rerio*)	Acute (96 h)	LC_{50}	> 500 (Direct Blue 15)	Brown 1992
Benzidine-based Cationic Indicators	Alga (*Pseudokirchneriella subcapitata*)	Chronic (14 days)	Population growth	10 (BB4)	Ericson 1977
Benzidine-based Cationic Indicators	Alga (*Anacystis aeruginosa*)	Chronic (1 day)	Population growth	2.5 (TTC)	Fitzgerald et al. 1952
Benzidine-based Cationic Indicators	Fish (*Petromyzon marinus*, *Ptychocheilus oregonensis*, *Oncorhynchus tshawytscha*, *Oncorhynchus kisutch*)	Acute (24 h)	LC_{50}	5–10 (TTC)	Applegate et al. 1957; MacPhee and Ruelle 1969

Benzidine-based Cationic Indicators	Fish (*Pimephales promelas*)	Acute (96 h)	LC_{50}	5.6 – > 5.6 (BB4)	Little and Lamb 1973, 1974

Abbreviations: EC_{50}, the concentration of a substance that is estimated to cause some effect on 50% of the test organisms; LC_{50}, the concentration of a substance that is estimated to be lethal to 50% of the test organisms

[a] Robust study summaries were conducted to determine the quality of the studies and are available upon request.

A limited number of empirical ecotoxicity studies are available for 3,3′-DMB and TODI (see Table 6-2 and divided up by substance in Appendix A, Table A-10).

Results indicate that both substances are generally expected to be moderately toxic to aquatic organisms, as indicated by a 72-hour EC_{50} (≥ 1.5–6.3 mg/L) in algae, a 48-hour EC_{50} (≥ 1.5–4.5 mg/L) in *Daphnia* and a 96-hour LC_{50} (0.25–13 mg/L) in fish. It is unclear whether the elevated aquatic toxicity value for fish (96-hour LC_{50} of 0.25 mg/L) for TODI (ECHA 2012) is driven by TODI or its corresponding amine 3,3′-DMB, since the substance hydrolyses rapidly when in contact with water. A chronic 21-day EC_{50} value of 0.64 mg/L for *Daphnia* (MITI 2000) for 3,3′-DMB is the most conservative empirical value available for the Benzidine Derivatives.

Table 6-2. Summary of empirical data for aquatic toxicity for Benzidine Derivatives

Subgroup	**Test organism**	**Type of test (duration)**	**Endpoint**	**Value (mg/L)**	**Reference**
Benzidine Derivatives (3,3′-DMB and TODI)	Alga	Chronic (72 h)	EC_{50}	≥ 1.5–6.3	ECHA 2012; MITI 2000
Benzidine Derivatives (3,3′-DMB and TODI)	*Daphnia*	Acute (48 h)	EC_{50}	≥ 1.5–4.5	Kuhn 1989[a]; ECHA 2012; MITI 2000
Benzidine Derivatives (3,3′-DMB and TODI)	*Daphnia*	Chronic (21 days)	EC_{50}	0.64	MITI 2000

Benzidine Derivatives (3,3′-DMB and TODI)	Fish	Acute (96 h)	LC_{50}	0.25–13	ECHA 2012; MITI 2000

Abbreviations: EC_{50}, the concentration of a substance that is estimated to cause some effect on 50% of the test organisms; LC_{50}, the concentration of a substance that is estimated to be lethal to 50% of the test organisms

[a] 24-hour EC_{50} (behaviour) = 3.2 mg/L for 3,3′-DMB.

No experimental toxicity data are available for the Benzidine-based Precursors or read-across substances.

6.1.2 Empirical Studies for Other Environmental Compartments

Toxicological data were reported for a formulated product, trypan blue, which contains 80% Direct Blue 14 (Hulzebos et al. 1993). Seven-day and 14-day EC_{50} growth studies resulted in moderate (bordering on high) toxicity values of 263 (196–352) µg/g of soil and 290 (250–337) µg/g of soil, respectively, for lettuce (*Lactuca sativa*) (Table 6-3).

No empirical data were available on the toxicity of Benzidine-based Dyes in sediment.

Table 6-3. Empirical soil toxicity data from representative substances in the Benzidine-based Direct Dyes subgroup

Subgroup	**Test organism**	**Type of test**	**Endpoint**	**Value (mg/kg of soil)[a]**	**Reference**
Benzidine-based Direct Dyes (Direct Blue 14)	Lettuce (*Lactuca sativa*)	Chronic (7 days)	EC_{50} (growth) (soil)	196–352 (263)	Hulzebos et al. 1993
Benzidine-based Direct Dyes (Direct Blue 14)	Lettuce (*Lactuca sativa*)	Chronic (14 days)	EC_{50} (growth) (soil)	250–337 (290)	Hulzebos et al. 1993

Abbreviation: EC_{50}, the concentration of a substance that is estimated to cause some effect on 50% of the test organisms

[a] Robust study summaries were conducted to determine the quality of the study and are available upon request.

No toxicity data were found on the toxicity of Benzidine Derivatives in soil or sediment. However, a study conducted by Chung et al. (1998) on the toxicity of benzidine congeners on 18 bacterial strains, including nitrogen-fixing *Azotobacter vinelandii*, determined that TODI, 3,3′-DMOB and 4N-TMB were not inhibitory to any of the bacterial species tested.

6.1.3 Modelled Results

Although empirical ecotoxicity data for the Benzidine-based Dyes and Benzidine-based Precursors were limited, predictions of ecotoxicity using (Q)SAR models are considered unreliable. Anionic dye classes are difficult to model because the properties of these dyes fall outside the domains of applicability of the available models and because of the error associated with estimation of log K_{ow} values used as input to the models.

However, due to their less complex chemical structure and a lower tendency to ionize, aquatic toxicity predictions for three Benzidine Derivatives, 3,3′-DMB, 3,3′-DMOB and 4N-TMB, were modelled using ECOSAR (2011), as they are considered to be within the model domain of applicability (Table 6-4).

Predictions for 4N-TMB were determined using the EVA corrected log K_{ow} value of 3.53. Model predictions are consistent with the empirical results available for 3,3′-DMB and TODI, indicating that these Benzidine Derivatives are expected to be moderately toxic to algae, *Daphnia* and fish.

Table 6-4. Summary of modelled data for aquatic toxicity for the Benzidine Derivatives

Substances	Test organism	Type of test	Endpoint	Value (mg/L)	Reference
3,3′-DMB and 3,3′-DMOB	Fish	Acute (96 h)	LC_{50}	9.06–31.91	ECOSAR 2011
3,3′-DMB and 3,3′-DMOB	Fish	Chronic (14 days)	LC_{50}	1.81–21.19	ECOSAR 2011
3,3′-DMB and 3,3′-DMOB	Fish	Chronic	ChV	0.017–0.092	ECOSAR 2011
3,3′-DMB and 3,3′-DMOB	*Daphnia*	Acute (48 h)	LC_{50}	1.26–2.22	ECOSAR 2011
3,3′-DMB and 3,3′-DMOB	*Daphnia*	Chronic	ChV	0.014–0.033	ECOSAR 2011

3,3′-DMB and 3,3′-DMOB	Alga	Acute (96 h)	EC_{50}	7.99–11.28	ECOSAR 2011
3,3′-DMB and 3,3′-DMOB	Alga	Chronic	ChV	4.55–6.87	ECOSAR 2011
4N-TMB	Fish	Acute (96 h)	LC_{50}	8.35	ECOSAR 2011
4N-TMB	Fish	Chronic (14 days)	LC_{50}	8.65	ECOSAR 2011
4N-TMB	Fish	Chronic	ChV	0.96	ECOSAR 2011
4N-TMB	*Daphnia*	Acute (48 h)	LC_{50}	5.90	ECOSAR 2011
4N-TMB	*Daphnia*	Chronic	ChV	0.87	ECOSAR 2011
4N-TMB	Alga	Acute (96 h)	EC_{50}	5.44	ECOSAR 2011
4N-TMB	Alga	Chronic	ChV	2.68	ECOSAR 2011

Abbreviations: ChV, chronic ecotoxicity end-point value; EC_{50}, the concentration of a substance that is estimated to cause some effect on 50% of the test organisms; LC_{50}, the concentration of a substance that is estimated to be lethal to 50% of the test organisms

Since no experimental data were available for Benzidine-based Precursors and they are at most only weakly ionized in the environment, modelled data for the neutral form are presented in Table 6-5 for Naphthol AS-BR and Table 6-6 for TCDB.

Note that warnings were received from ECOSAR (2011), CPOPs (2008) and ACD/I-Lab (2010-2011) for the majority of model output values because most toxicity values were in excess of the minimum of the range of estimated water solubilities for Naphthol AS-BR (8.97×10^{-6} mg/L) and TCDB (0.26 mg/L). The baseline toxicity model was chosen for ECOSAR over other structure–activity relationships (SARs) such as Amides, Phenylamines and Polyphenols, since it has the most chemicals in the training set and widest range of log K_{ow} values. This is important for these two substances, since they both have high modelled log K_{ow} values near the edge of the model domains. Given that no experimental data are available and that the toxicities are predicted using modelled log K_{ow} data, this information should be considered with a high degree of uncertainty in the overall weight of evidence for toxicity. This is demonstrated by the 5 orders of magnitude toxicity range for Naphthol AS-BR.

Table 6-5. Summary of modelled data for aquatic toxicity of Naphthol AS-BR using ACD/I-Lab (2010-2011), CPOPs (2008) and the baseline toxicity model from ECOSAR (2011)

Test organism	Type of test (duration)	Endpoint	Value (mg/L)	Reference
Fish	Chronic	ChV	0.000 51[a]	ECOSAR 2011
Fish	Acute (96 h)	LC_{50}	0.006[a]	ECOSAR 2011
Fish	Acute	LC_{50}	7.3[a,b]	ACD/I-Lab 2010–2011
Fish	Acute	LC_{50}	0.29	CPOPs 2008
Daphnid	Chronic	ChV	0.0018[a]	CPOPs 2008
Daphnid	Acute (96 h)	LC_{50}	0.007[a]	ECOSAR 2011
Daphnid	Acute	LC_{50}	0.000 89[a,c]	ECOSAR 2011
Daphnid	Acute	EC_{50}	0.19[a]	ACD/I-Lab 2010–2011
Daphnid	Acute	LC_{50}	0.16[a]	CPOPs 2008
Green alga	Chronic	ChV	0.033[a]	ECOSAR 2011
Green alga	Acute (96 h)	EC_{50}	0.032[a]	ECOSAR 2011

Abbreviations: ChV, chronic ecotoxicity end-point value; EC_{50}, the concentration of a substance that is estimated to cause some effect on 50% of the test organisms; LC_{50}, the concentration of a substance that is estimated to be lethal to 50% of the test organisms

[a] Data in excess of minimum from range of predicted water solubilities for Naphthol AS-BR (8.97×10^{-6} mg/L).

[b] Not reliable in ACD/I-Lab (2010–2011)

[c] Borderline reliability in ACD/I-Lab (2010–2011)

Table 6-6. Summary of modelled data for aquatic toxicity of TCDB using ACD/I-Lab (2010–2011), CPOPs (2008) and the baseline toxicity model from ECOSAR (2011)

Test organism	Type of test (duration)	Endpoint	Value (mg/L)	Reference
Fish	Chronic	ChV	0.054	ECOSAR 2011
Fish	Acute (96 h)	LC_{50}	0.61[a]	ECOSAR 2011
Fish	Acute	LC_{50}	2.9[a,b]	ACD/I-Lab 2010–2011
Fish	Acute	LC_{50}	≤ 0.31[a]	CPOPs 2008
Daphnid	Chronic	ChV	0.083	ECOSAR 2011
Daphnid	Acute (96 h)	LC_{50}	0.52[a]	ECOSAR 2011
Daphnid	Acute	LC_{50}	0.026[c]	ACD/I-Lab 2010–2011
Daphnid	Acute (48 h)	EC_{50}	≤ 0.87[a]	CPOPs 2008
Daphnid	Acute (48 h)	LC_{50}	≤ 0.19	CPOPs 2008
Green alga	Chronic	ChV	0.51[a]	ECOSAR 2011
Green alga	Acute (96 h)	EC_{50}	0.80[a]	ECOSAR 2011

Abbreviations: ChV, chronic ecotoxicity end-point value; EC50, the concentration of a substance that is estimated to cause some effect on 50% of the test organisms; LC50, the concentration of a substance that is estimated to be lethal to 50% of the test organisms

a Data in excess of minimum from range of predicted water solubilities for TCDB (0.26 mg/L).

b Not reliable in ACD/I-Lab (2010–2011)

c Borderline reliability in ACD/I-Lab (2010–2011)

6.1.4 Carcinogenicity and Mode of Action

Limited information is available on the environmental carcinogenicity of benzidine-based dyes. As mentioned previously, cleavage of the azo bond under anaerobic or reducing conditions (e.g., deep layers of sediments) is known to result in aromatic amines, some of which are known to be potentially carcinogenic. Studies using Direct Black 38 have shown breakdown to benzidine and 4-aminobiphenyl, which have been found to be mutagenic and carcinogenic substances (Isik and Sponza 2004; Bafana et al. 2009a; IARC 2010e) and acutely toxic (LC_{50} < 1 mg/L) to some crustaceans and juvenile fish (Øllgaard et al. 1998). However, because they are formed only in deep anoxic sediment, there is a lower likelihood that aquatic organisms will be exposed to these more harmful metabolites. Limited information was available for terrestrial organisms. The potential genotoxicity and carcinogenic effects of Benzidine Derivatives to sediment-dwelling biota remain a source of uncertainty.

Limited data are available on the deoxyribonucleic acid (DNA) and/or protein binding of Benzidine-based Substances and Benzidine Derivatives in the environment. All 44 Benzidine-based Substances and Benzidine Derivatives were profiled for structural alerts using the OECD QSAR Toolbox for DNA interactions and protein binding. These mechanistic-based profilers identify the potential of substances to cause genotoxicity or protein binding–related effects (e.g., chromosomal aberrations, acute inhalation toxicity and acute aquatic toxicity). Possible DNA binding was identified through nitrenium ion formation for 33 (18 Benzidine-based Direct Dyes, 9 Benzidine-based Acid Dyes, 2 Benzidine-based Cationic Indicators and 4 Benzidine Derivatives) of the 44 Benzidine-based Substances. Five substances (2 Benzidine-based Acid Dyes, Benzidine-based 1 Direct Dye, 1 Benzidine-based Precursor and 1 Benzidine Derivative) have structural alerts suggesting that they are capable of directly acylating proteins resulting in adduct formation, while the remaining 39 substances showed no protein binding potential.

These profiling results indicate that 33 of these substances may exert additional toxicity (mostly DNA binding and in some cases protein binding) to the standard narcotic response. These data suggest only a potential for these adverse effects to occur and do not confirm that they will occur. Consequently, the profiling results will be considered as a line of evidence in determining the overall potential for adverse effects that these substances may elicit. However, the profiling results are consistent with the moderate, bordering on high, acute aquatic toxicity results observed for some of the subgroups addressed in this assessment. This suggests that given significant bioavailability in the environment, the reactivity of these compounds can result in acute aquatic toxicity above the baseline of narcosis (as correlated with log K_{ow}). The potential genotoxicity and carcinogenicity of these substances for human relevance are addressed in the Potential to Cause Harm to Human Health section.

6.1.5 Derivation of the PNEC and Rationalization of the Assessment Factor

Benzidine-based Acid Dyes

Due to the paucity of toxicity data, a grouping approach was used in the development of the predicted no-effect concentration (PNEC) for the Benzidine-based Acid Dyes subgroup. Toxicity data for Acid Red 114 were chosen as read-across for Benzidine-based Acid Dyes since Acid Red 114 shares chemical structural similarity with other members of the group as well as common physical and chemical properties as part of the same application class and because reliable toxicity information was available.

The 96-hour LC_{50} of 4 mg/L for common carp (MITI 1992) was selected as the critical toxicity value (CTV) because it was the most sensitive valid experimental value. A PNEC was derived by dividing the CTV by an assessment factor of 100 (to account for interspecies and intraspecies variability in sensitivity and to estimate a long-term no-effects concentration from a short-term LC_{50}) to give a value of 0.04 mg/L.

Benzidine-based Direct Dyes

A grouping approach was also used in the development of the PNEC for the Benzidine-based Direct Dyes subgroup. Direct Blue 14 was chosen as the surrogate or structural analogue for read-across, given that it is the most representative for the grouping (i.e., 70–100% structural similarity with most substances according to the OECD QSAR Toolbox), shares similar physical and chemical properties as part of the same application class and has the most toxicity information available.

The CTV selected was the 96-hour LC_{50} of 11.3 mg/L for freshwater shrimp (*Desmocaris trispinosa*; Ogugbue and Oranusi 2006), as this was the most sensitive valid experimental value. The aquatic PNEC was then derived by dividing this value by an assessment factor of 100 (to account for differences in interspecies and intraspecies variability and to estimate a long-term no-effects concentration from a short-term LC_{50}). Therefore, a PNEC of 0.11 mg/L was calculated for the Benzidine-based Direct Dyes.

The soil CTV was the 7-day EC_{50} (growth) for lettuce (*Lactuca sativa*) of 263 mg/kg soil (Hulzebos et al. 1993), as this was the most sensitive value from the sole experimental study. The soil PNEC was then derived by dividing this value by an assessment factor of 100 (to account for interspecies and intraspecies variability in sensitivity and the fact that this is the only study available, and because the 7-day test length may be considered intermediate between short and long term for vascular plants). The result of the adjustment is a calculated value of 2.63 mg/kg soil.

This soil PNEC will also be used in the weight of evidence for Benzidine-based Acid Dyes, since there is a paucity of soil toxicity data and because direct and acid dyes share similar physical and chemical properties.

Benzidine-based Cationic Indicators

The aquatic CTV selected was the 96-hour LC_{50} of 5.6 mg/L for fathead minnow (*Pimephales promelas*) using the structural analogue BB4 (Little and Lamb 1973), as this was the most valid sensitive experimental value. A PNEC was derived by dividing the CTV by an assessment factor of 100 (to account for interspecies and intraspecies variability in sensitivity and to estimate a long-term no-effects concentration from a short-term 96-hour LC_{50}) to give a value of 0.056 mg/L. This fish toxicity endpoint value was chosen for Benzidine-based Cationic Indicators since few data are available and there are similarities between BB4 and the Benzidine-based Cationic Indicators grouping due to their sharing of common functional groups and certain physical and chemical properties.

Benzidine-based Precursors

A PNEC for Naphthol AS-BR was derived from the chronic toxicity value of 0.00051 mg/L using the baseline toxicity model as the most sensitive for fish, which is within 1 order of magnitude of the substance’s mean predicted water solubility. An assessment factor was not used, since the CTV is a very conservative modelled chronic value that is driven by a very high modelled log K_{ow}, which is also conservative.

A separate PNEC for TCDB was derived from the chronic modelled toxicity value of 0.083 mg/L for *Daphnia* as the most sensitive value that is below the lowest predicted water solubility. An assessment factor of 10 was used to account for interspecies and intraspecies variability in sensitivity, to give a PNEC value of 0.0083.

Benzidine Derivatives

Based on available experimental and modelled data, Benzidine Derivatives share similar ecological toxicity, which may be due to common modes of action (due to the presence of benzenamine or aniline groups). Therefore, the most sensitive valid experimental value for 3,3′-DMB was selected as the CTV and used as read-across to define the Benzidine Derivatives subgroup.

The CTV selected was the chronic 21-day EC_{50} immobilization value of 0.64 mg/L for *Daphnia* (MITI 2000) for 3,3′-DMB. A PNEC was derived for the Benzidine Derivatives by dividing this value by an assessment factor of 10 (to account for interspecies and intraspecies variability in sensitivity) to yield a value of 0.064 mg/L.

6.1.6 Ecological Effects Summary

Based on lines of evidence involving empirical and read-across aquatic ecotoxicity data, it may be concluded that Benzidine-based Acid and Direct Dyes as well as Benzidine-based Cationic Indicators may be expected to cause harm to aquatic organisms at low concentrations. Based on limited empirical soil ecotoxicity data, Benzidine-based Acid and Direct Dyes as well as Benzidine-based Cationic Indicators are not expected to cause harm to soil-dwelling organisms at low concentrations.

In addition, based on various lines of evidence involving empirical and modelled ecotoxicity data in various environmental compartments, it may be concluded that Benzidine Derivatives may be expected to cause harm to aquatic organisms at low concentrations. Data for soil- and sediment-dwelling organisms are not available.

Finally, based on various lines of evidence involving empirical ecotoxicity data in various environmental compartments, it may be concluded that Benzidine-based Precursors may cause harm to aquatic organisms at low concentrations. Data for soil- and sediment-dwelling organisms are not available.

6.2 Ecological Exposure Assessment

6.2.1 Releases to the Environment

No measured concentrations of substances in the Benzidine-based Acid and Direct Dyes, Benzidine-based Cationic Indicators, Benzidine-based Precursors or Benzidine Derivatives subgroups in the Canadian environment have been identified. Environmental concentrations have therefore been estimated from available information.

Anthropogenic releases of a substance to the environment depend on various losses that occur during the manufacture, industrial use, consumer/commercial use and disposal of the substance. In order to estimate releases to the environment occurring at different stages of the life cycle of the substances evaluated in this Screening Assessment, Environment Canada compiled information on the sectors and product lines relevant to the substances. In addition, an effort was made to compile emission factors[4] for emissions to wastewater, land and air at different life cycle stages. Relevant factors were considered, uncertainties were recognized and assumptions were made, when necessary, during each stage, depending on information available.

The information compiled was to give an overview of the potential losses occurring at different stages of the life cycle and the receiving media involved, as well as to identify the life cycle stages that are likely the largest contributors to the overall environmental concentrations. Recycling activities and transfer to waste disposal sites (landfill, incineration) were also considered. However, unless specific information on the rate of or potential for release from landfills and incinerators was available, releases to the environment from disposal were not quantitatively accounted for.

This information was used to further develop exposure scenarios for the purpose of determining PECs.

6.2.2 Ecological Exposure

The general methodology used for characterizing ecological exposure to the Benzidine-based Dyes is to evaluate the highest release sector or use first and then determine if it is necessary to provide the same level of detail for other sectors or uses. If the highest release sector or use is determined to present low ecological concern, then all other sectors or uses would be judged to also be of low concern due to their lower releases and would therefore not be analyzed further. Otherwise, these other sectors or uses will be examined to determine the extent of the ecological concern.

Benzidine-based Dyes are used in various sectors, based on reported uses in the literature (e.g., Hunger 2003); however, the dominant sector for Benzidine-based Dyes is the textile and leather sector, as discussed in the Uses section of this report.

The textile wet processing sector was selected as the scenario resulting in highest exposure for the assessment of the ecological risks posed by Benzidine-based Dyes. The textile sector was chosen, as it is expected to be the major use sector for

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[4] An emission factor is generally expressed as the fraction of a substance released to a given medium, such as wastewater, land or air, during its 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 and monitoring data.

Benzidine-based Dyes, and the fraction of dyes lost to wastewater during textile dyeing operations is higher than that from all other use sectors. The laundering of textile products as well as textile wet processing are identified as major uses. During laundering of textile products, dyes can be lost to wastewater. The loss is estimated to be 2.5–5% by dividing the maximum 10% loss over the lifetime of textile products (Øllgaard et al. 1998) by a typical life expectancy of 2–4 years for textile products (Michigan Institute of Laundering and Dry Cleaning 2012). The average loss to wastewater from textile wet processing is reported to be 10% for acid dyes and 12% for direct dyes (OECD 2004), significantly higher than the 2.5–5% loss from the laundering of textile products. Higher releases are therefore expected from the textile wet processing sector.

Based on the reported uses from surveys and the literature, the textile wet processing sector was determined to be an important use sector for the Benzidine-based Acid and Direct Dyes. A quantitative exposure assessment based on the textile wet processing sector was chosen for the Benzidine-based Acid Dyes, because this was the only reported use from industry surveys conducted for the years 2005 and 2006 (Canada 2006b, 2008b). Although these surveys did not reveal the specific application of the Benzidine-based Direct Dyes within the textile sector, literature data indicate that they exhibit high affinity for cellulose fibres and are applied directly to cellulose-containing textiles (ETAD 1995b; Hunger 2003).

Exposure Scenario #1: Aquatic Exposure via Potential Releases from Textile Wet Processing Mills

The approach for estimating aquatic exposure for Benzidine-based Acid and Direct Dyes is to focus on textile wet processing as the major release sector and to evaluate their aquatic PECs from individual sites.

In total, 145 textile wet processing mills were identified in a survey conducted by Environment Canada in 1999 (Crechem 1998). Those mills included six different types (knit, woven, stock/yarn, wool, carpet and non-woven) and eight facilities whose types were unknown. The majority of these mills discharged their wastewaters to municipal wastewater treatment systems before reaching the aquatic environment.

An effort was made to obtain site-specific conditions needed for aquatic exposure calculations. For this purpose, the 145 mills were examined together with their local wastewater treatment systems and receiving water bodies. For 75 mills, information was available on wastewater treatment effluent flow and receiving river flow. For the remaining 70 mills, there was a lack of accurate information on local municipal wastewater treatment systems or their receiving water bodies. The 75 mills, however, cover all major mill types (knit, woven, non-woven, stock/yarn, wool and carpet) and are believed to provide sufficient representation of the textile dyeing operations conducted by the sector.

The aquatic PECs for the 75 mills were determined at 33 sites, based on an estimated amount of the Benzidine-based Acid or Direct Dyes used. This estimated amount is associated with any given single mill and determined from the highest quantity sold to one single textile mill from the CEPA 1999 section 71 survey data.

Other parameters considered in the calculation of aquatic PECs include the amount of the Benzidine-based Acid or Direct Dyes released from textile dyeing operations and removed by wastewater treatment systems, as well as the dilution by the receiving water. Since the use and release of the Benzidine-based Acid or Direct Dyes are intermittent, additional dilution by lagoons is also considered. The lagoon dilution is warranted by the fact that lagoons have much longer hydraulic retention times than the release durations for the Benzidine-based Acid or Direct Dyes. This additional dilution is not considered valid for primary or secondary wastewater treatment systems due to their relatively short hydraulic retention times.

The aquatic PEC results for the 75 mills are presented in Table 12 in the section on Characterization of Ecological Risk, and the calculations are explained in Appendix B. The results show that the aquatic PECs range from 0.05 to 24 µg/L for the Benzidine-based Acid Dyes and from 0.06 to 20 µg/L for the Benzidine-based Direct Dyes. These values are considered conservative because they are based on a maximum quantity of the Benzidine-based Acid or Direct Dyes used at each single mill and zero removal from on-site wastewater treatment.

Exposure Scenario #2: Soil Exposure via Biosolids Application to Land

Soil exposure to the Benzidine-based Acid or Direct Dyes in this Screening Assessment was estimated using a conservative scenario. In this scenario, biosolids produced from wastewater treatment systems at the 33 sites evaluated for the aquatic exposure were assumed to be applied to land at the maximum allowable rate over a substantial number of years. The scenario also assumed that the Benzidine-based Acid or Direct Dyes accumulated in soil do not incur any losses from degradation, volatilization, soil runoff or leaching. This conservative scenario yielded a soil PEC of 0.18 mg/kg for the Benzidine-based Acid Dyes and 0.24 mg/kg for the Benzidine-based Direct Dyes (see Appendix C for detailed calculations).

6.3 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 conservative risk quotient analyses, which are outlined below.

6.3.1 Benzidine-based Acid and Direct Dyes

Aquatic Risk Quotient Analysis

A risk quotient analysis, integrating conservative estimates of exposure with toxicity information, was performed for the aquatic medium to determine whether there is potential for ecological harm in Canada. The analysis was conducted for a set of 75 mills at 33 sites and is considered representative of the textile sector.

Sector-based site-specific industrial scenarios (considering the actual receiving water body) show PECs of 0.05–24 µg/L for Benzidine-based Acid Dyes and 0.06–20 µg/L for Benzidine-based Direct Dyes (Environment Canada 2012). PNECs of 0.04 and 0.11 mg/L were derived for Benzidine-based Acid and Direct Dyes, respectively (see the Ecological Effects Assessment section). The resulting risk quotients (PEC/PNEC) are 0.0013–0.6 for Benzidine-based Acid Dyes and 0.00055–0.16 for Benzidine-based Direct Dyes. These results show low concern in the aquatic compartment from the use of the two groups of dyes in textile dyeing. Therefore, harm to aquatic organisms is unlikely at these sites, due in part to the large dilution capacities of the locations.

Soil Risk Quotient Analysis

Risk quotients for soil were also determined by dividing the PECs by the PNEC (2.63 mg/kg). The soil risk quotient for Benzidine-based Acid Dyes was 0.068, while the soil risk quotient for Benzidine-based Direct Dyes was 0.091. These risk quotients are conservative estimates, since many conservative assumptions were used in their derivation. The major conservative assumptions used include a) maximum quantities of acid or direct dyes based on the upper limit of each individual dye reported by industry via regulatory surveys; b) no deduction of the quantities of acid or direct dyes that are released to lagoons and do not end up in land-applied biosolids; and c) no loss assumed for the dyes present in soil via degradation, soil runoff or leaching over 10 years of biosolids application.

The PECs for the Benzidine-based Acid and Direct Dyes in water and soil show that they are well below the PNECs. Since all other sectors are expected to have lower releases, they are not likely to present ecological concern and are therefore not given further exposure analysis.

Benzidine-based Cationic Indicators, Benzidine-based Precursors and Benzidine Derivatives

No recent commercial data have been submitted for Benzidine-based Cationic Indicators, Benzidine-based Precursors or Benzidine Derivatives, and stakeholder interest has been declared for only one Benzidine Derivative. This is consistent with an understanding that the commercial use of benzidine-based dyes are also decreasing due to restrictions in several jurisdictions. While these substances may be used in commerce in Canada below survey reporting thresholds of 100 kg/year, or, in the case of the Benzidine Derivatives, transformation products may be formed through azo bond cleavage, environmental concentrations are expected to be very low and dispersive. While the estimated PNECs for these groups of substances differ, given the lack of data on commercial activity and the assumption that releases are low, no significant ecological risk from exposure is expected.

6.3.2 Discussion of Weight of Evidence and Conclusion of Ecological Risk Characterization

The approach taken in this ecological screening assessment was to examine various supporting information and develop conclusions based on a weight of evidence approach and using precaution as required under CEPA 1999. Lines of evidence considered include results from a conservative risk quotient calculation, as well as information on persistence, bioaccumulation, ecological effects, sources, fate of the substance and presence and distribution in the environment. Various lines of evidence for each subgrouping are summarized below, along with relevant uncertainties leading to overall conclusions.

Benzidine-based Acid and Direct Dyes are anthropogenically produced and not expected to occur naturally in the environment. The majority of the Benzidine-based Acid Dyes and Benzidine-based Direct Dyes in this grouping were not found to be in commerce according to recent surveys. No data concerning concentrations of these substances in the Canadian environment have been identified. Benzidine-based Acid and Direct Dyes are complex anionic molecules that have relatively high water solubility (greater than 1 g/L) and are expected to dissociate at environmentally relevant pH levels. Since there is a relative paucity of data, Benzidine-based Acid Dyes were grouped together due to their similar physical and chemical properties as well as relatively similar chemical structures (e.g., sharing common functional groups, but varying in number). Also, log K_{ow} data from azo-based acid dyes were used as read-across for the Benzidine-based Acid Dyes. Due to their high water solubility and affinity for oppositely charged organic particles, Benzidine-based Acid and Direct Dyes are expected to be found in water, sediment and soil. Given very low expected vapour pressures and Henry’s Law constants, 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, Benzidine-based Acid and Direct Dyes have low experimental log K_{ow} values (0.42 and 0.77, respectively). Estimated and experimental log K_{ow} values were compared with experimental BCFs for fish for a number of dyes (Anliker et al. 1981; Øllgaard et al. 1998; ETAD 1995b). The reported BCF values for Benzidine-Based Acid and Direct Dyes were low, ranging from 42 to 84, indicating that these dyes are not likely to bioconcentrate in aquatic organisms. Furthermore, Benzidine-based Acid and Direct Dyes are not expected to bioconcentrate due to their high molecular weights (> 600 g/mol) and relatively large minimum and maximum effective cross-sectional diameters, which suggest slow uptake potential. Bioaccumulation resulting from exposures of organisms to these substances in soil and sediment is not well understood due to minimal and limited data, in large part due to the high water solubility of these substances. According to empirical and modelled data, Benzidine-based Acid and Direct Dyes are expected to biodegrade very slowly in aerobic environments and are therefore considered to be persistent in water, sediment and soil. However, Benzidine-based Acid and Direct Dyes may degrade and transform to certain benzidine derivatives if they reach anaerobic environments. A source of uncertainty is the potential carcinogenicity and mutagenicity of these benzidine derivatives and their potential effects on sediment-dwelling biota. Based on lines of evidence involving empirical specific and read-across aquatic and terrestrial ecotoxicity data, it may be concluded that Benzidine-based Acid and Direct Dyes may be expected to cause harm to aquatic organisms at moderately low concentrations (4 and 11 mg/L, respectively). Toxicity data are limited for the terrestrial environment and unavailable for sediment-dwelling organisms. A conservative exposure analysis of the textile sector was done because that sector was anticipated to present the highest potential ecological risk related to industrial releases to the environment for these substances. Risk quotients calculated from PECs for Benzidine-based Acid and Direct Dyes released from textile wet processing mills and PNECs in the aquatic environment as well as risk quotients calculated from PECs for the application of biosolids containing Benzidine-based Acid and Direct Dyes to soil and PNECs in the soil environment show that at conservatively predicted levels of release, Benzidine-based Acid and Direct Dyes are not likely to result in significant aquatic or soil exposure. Overall, the results of this assessment lead to the conclusion that Benzidine-based Acid and Direct Dyes have a low potential to cause ecological harm in Canada.

Benzidine-based Cationic Indicators are also anthropogenically produced and not expected to occur naturally in the environment. Neither of the two Benzidine-based Cationic Indicators was identified to be in commerce above the reporting thresholds according to recent surveys, nor were Canadian monitoring data found. Benzidine-based Cationic Indicators also have relatively high water solubility (approximately 9–10 mg/L) and are expected to dissociate at environmentally relevant pH levels. These two substances were found to have similar physical and chemical properties and chemical structures, so any data available for either was shared. Also, read-across data from an azo-based cationic dye analogue and the substance TTC, which contains the tetrazolium chloride moiety, were used for ecotoxicity. Since Benzidine-based Cationic Indicators have a high water solubility and affinity for negatively charged organic particles, they are expected to be found in water, sediment and soil, but not air. Since

Benzidine-based Cationic Indicators are charged, they have low experimental log K_{ow} values. While BCFs were not available for these substances, they are similar enough to Benzidine-based Acid and Direct Dyes in their physical and chemical properties to indicate that these dyes are not likely to bioconcentrate in aquatic organisms. Data on terrestrial bioaccumulation and in sediment are minimal and limited, in large part due to the high water solubility of these substances. Benzidine-based Cationic Indicators are expected to biodegrade very slowly in aerobic environments and are therefore considered to be persistent in water, sediment and soil. Based on lines of evidence involving empirical specific and read-across aquatic and terrestrial ecotoxicity data, it may be concluded that Benzidine-based Cationic Indicators may be expected to cause harm to aquatic organisms at moderately low concentrations (pivotal value of 5.6 mg/L). Toxicity data are unavailable for the terrestrial environment and for sediment. However, given that Benzidine-based Cationic Indicators are not known to be in commerce in Canada, it is not believed that they would have a harmful effect on the environment.

The Benzidine-based Precursors Naphthol AS-BR and TCDB are anthropogenically produced, not expected to occur naturally in the environment and not found to be in commerce above the reporting thresholds in recent surveys. Naphthol AS-BR is sparingly soluble and not expected to dissociate under environmentally relevant pH levels, while TCDB is moderately soluble and is expected to dissociate readily. These two substances were addressed separately, since their physical and chemical properties as well as chemical structures are very different. Models were used to predict most results, given that read-across data from other substances could not be used, as no close analogues with data could be found. Since Naphthol AS-BR has very limited solubility, it is expected to reside mostly in soil or sediment if released to the environment, while TCDB is expected to stay in the water column or bind with particles, given its acidic character. Both Benzidine-based Precursors have moderate to high estimated log K_{ow} values, and experimental BCFs were not available for these substances. Modelled aquatic BCFs were moderate to high for TCDB and Naphthol AS-BR. Benzidine-based Precursors are expected to biodegrade very slowly in aerobic environments and are therefore considered to be persistent in water, sediment and soil. Based on lines of evidence involving two sets of modelled aquatic toxicity data for acute and chronic endpoints, it may be concluded that both TCDB and Naphthol AS-BR may be expected to cause harm to aquatic organisms at low concentrations (pivotal values < 1 mg/L). Toxicity data are unavailable for the terrestrial environment and for sediment. However, given that TCDB and Naphthol AS-BR are not known to be in commerce in Canada, it is not likely that either substance would have a harmful effect on the environment.

Benzidine Derivatives are low molecular weight (212–285 g/mol) substances that contain biphenyl. These anthropogenic substances are not expected to occur naturally in the environment, and none were found to be in commerce above the reporting thresholds in Canada according to recent surveys. Benzidine Derivatives have primarily been used as intermediates for the synthesis of colorants, but may also be used to a lesser degree to manufacture other chemicals (HSDB 1983–). No data concerning concentrations of these substances in the Canadian environment have been identified. The Benzidine Derivatives are generally moderately soluble (greater than 10 mg/L), have low to moderate log K_{ow} values (1.7–3.5) and will become ionic at low pH levels, as indicated by their pK_a values (3.4–4.7). Based on these properties, as well as their high potential for binding to particulate matter and sediment (see Environment Canada and Health Canada 2013), Benzidine Derivatives are expected to be found in water, sediment and soil. Some Benzidine Derivatives may be considered semi-volatile; however, they are unlikely to stay in air if released to this compartment. Therefore, long-range transport is not anticipated to be of concern. This is further confirmed by calculated persistence data indicating that Benzidine Deratives are expected to degrade rapidly in air (Meylan and Howard 1993; AOPWIN 2010. However, other empirical and modelled data indicate that Benzidine Derivatives biodegrade slowly in aerobic environments and are considered to be persistent in water, sediment and soil (Kawasaki 1980; MITI 1992; ECHA 2012). Moderate to high water solubility, low to moderate log K_{ow} values as well as low empirical BCFs (4.8–47) for 3,3′-DMB (MITI 1992) indicate that Benzidine Derivatives will not bioconcentrate in aquatic organisms. Empirical and modelled data indicate that Benzidine Derivatives are moderately toxic to fish and algae (> 1 mg/L), but may be highly toxic to invertebrates (< 1 mg/L) (Kuhn 1989; ECHA 2012; MITI 2000; ECOSAR 2011). However, given that releases of Benzidine Derivatives to the aquatic compartment as well as to other media are expected to be minimal based on their non-commercial status, it is not believed that Benzidine Derivatives would have a harmful effect on the environment.

6.3.3 Uncertainties

In general, with the exception of certain Benzidine Derivatives, substances addressed in this report had limited data available. As a result, a read-across approach using data from selected analogues was the best alternative to estimating physical and chemical properties. In the case of Benzidine-based Precursors, only modelled data could be used.

This paucity of information necessitated the generation of model predictions for biodegradation and inference of bioaccumulative potential using available data on physical and chemical properties. Certain empirical physical, chemical and toxicological data were generated with formulated products. Therefore, when possible, available data from relevant analogues were also used to inform the read-across groupings. 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. The use of assessment factors in determining a PNEC is intended to address these uncertainties. While the soil and sediment exposure media were found to be important for Benzidine-based Acid and

Direct Dyes, as well as Benzidine-based Cationic Indicators, effects data were not generally available.

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 as well as sediment and soil. Conservative assumptions were made when using models to estimate concentrations in receiving water bodies, sediment and soil.

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 textile sector exposure analysis.

7. Potential to Cause Harm to Human Health

The human health assessment for Benzidine-based Dyes and Related Substances focuses on substances that are in commerce (based on information received in response to the section 71 survey) and/or for which available information indicates potential exposure to the general population of Canada: 3,3'-DMB and Acid Red 97. Direct Blue 14 is also in commerce based on available information (Environment Canada 2009; DPD 2012), but reported uses of this substance do not result in exposure for the general population in Canada.

7.1 Exposure Assessment

7.1.1 Environmental Media

As described in the section on Sources, limited quantities of Acid Red 97 and Direct Blue 14 were reported to be in commerce. Based on the uses of these substances and the overall low volumes of Benzidine-based Substances or Benzidine Derivatives in commerce, exposure from environmental media is not expected.

As none of the substances in this assessment were identified to be used in food packaging or food-related applications, these sources are not considered to contribute to exposure to Benzidine-based Substances or Benzidine Derivatives.

7.1.2 Consumer Products

In general, Benzidine-based Dyes are used predominantly for dyeing textiles, Benzidine-based Cationic Indicators as laboratory reagents and Benzidine-based Precursors and Benzidine Derivatives as intermediates in the manufacturing of dyes, pigments, colouring agents and chemicals.

To characterize potential general population exposure to Benzidine-based Substances and Benzidine Derivatives from contact with products, the following information was taken into consideration: information received from section 71 surveys; information on the use of these substances and their presence in products based upon publicly available sources; information submitted to Health Canada (e.g., pursuant to the *Food and Drugs Act*); recent data from testing of products on the Canadian market; and market surveillance/monitoring data from Europe and Japan.

Leaching of 3,3'DMB from black polyamide cooking utensils was reported in an Irish study (McCall et al. 2012). Acid Red 97 is identified to be used primarily for dyeing textiles and leather (CII 2011). Direct Blue 14 was reported to be used in medical devices and research and development (Environment Canada 2008); this substance is not present in consumer products and therefore exposure of the general population to this substance is not expected. The presence of other Benzidine-based Substances or Benzidine Derivatives in products, in particular in textiles, in Canada has not been identified.

Benzidine Derivatives

In the study conducted in Ireland (McCall et al. 2012), 84 black polyamide cooking utensils (e.g., spatulas, slotted spoons) purchased from various retail locations were analyzed for release of primary aromatic amines, including 3,3′-DMB, during a simulated use scenario. The contact area of the utensil was immersed in 3% acetic acid simulant solutions and left for 2 hours at 100°C. This was repeated two additional times to simulate repeated use. The results of the study showed leaching of 3,3′-DMB from 16 cooking utensils; the median, average and maximum concentrations from the third extraction were 1.4 µg/kg food, 3.9 µg/kg food and 30 µg/kg food, respectively. The authors indicated that primary aromatic amines leached from these utensils, under these test conditions, primarily due to incomplete polymerization. Significant variation in leaching levels was observed from identical utensils and among different types of cooking utensils.

Since the frequency of detection of 3,3′-DMB was less than 20%, the use of the median concentration was considered an appropriate metric for use in characterizing potential exposure of the general population. Using the median leaching level of 3,3′-DMB (1.4 µg/kg food) under acidic and elevated temperature, conservative estimates of potential exposure from the use of polyamide cooking utensils were derived to range from 0.002 µg/kg-bw per day (12 years of age and older) to 0.0065 µg/kg-bw per day (toddlers 0.5–4 years of age). See Appendix D for derivation of the exposure estimates.

Testing of products on the Canadian market conducted by Health Canada (2013) did not identify any detectable levels of 3,3′-DMB or 3,3′-DMOB in imported and domestic textile and leather products in Canada (limits of detections of 1.9 ppm for 3,3′-DMB and 1.5 ppm for 3,3′-DMOB). The investigation tested 66 samples of imported and domestic textile and leather products for EU22 aromatic amines[5] which are regulated under the EU22 regulation (EU 2006; Environment Canada and Health Canada 2013). The testing focused on children's toys, leather slippers, children's clothing and woollen items purchased in retail stores in Ottawa, Ontario, in August 2012 (Health Canada 2013). The testing procedure followed that of EU Standard BS EN 14362-1:2012 for testing EU22 aromatic amines (ECS 2012) and had a limit of quantification (LOQ) of 5.6 parts

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

per million (ppm) for 3,3′-DMB and 4.4 ppm for 3,3′-DMOB. Results are consistent with the global phaseout of EU22 aromatic amines and the corresponding azo dyes due to restrictions in other countries (Environment Canada and Health Canada 2013). Combined with no manufacture and import activities above the reporting threshold of 100 kg for these substances reported in response to section 71 surveys, direct and prolonged exposure to 3,3′-DMB and 3,3′-DMOB for the general population of Canada from contact with textile and leather is not expected.

Notwithstanding the restrictions in other countries, the presence of some EU22 aromatic amines has been reported in other jurisdictions. Compliance reports in Europe, the RAPEX alert system (RAPEX 2012) and the EurAzos project (EurAzos 2007), as well as a recent Japanese market survey (Kawakami et al. 2010), show the presence of 3,3′-DMOB and 3,3′-DMB in some textile, clothing and leather products, some of which were reported to be imported from other countries.

RAPEX is the EU rapid alert system shared by EU member states that facilitates the rapid exchange of information on products posing a serious risk to the health and safety of consumers. The operational procedures for RAPEX are described within the EU Product Safety Directive 2001/95/EC (EU 2001), which imposes a general safety requirement on any product put on the market for consumers. The EU22 aromatic amines listed in Appendix 8 of Regulation (EC) No 1907/2006 (EU 2006) are monitored by the RAPEX alert system (RAPEX 2012). A search of the RAPEX database for alert notifications made from 2010 to 2012 identified notifications for 5 and16 textile articles containing 3,3′-DMB and 3,3′-DMOB, respectively; concentrations ranged from 5.16 to 640 mg/kg for 3,3′-DMB and from 8.7 to 615 mg/kg for 3,3′-DMOB based on standardized test methods (see Table 7-1).

The total number of products tested is unknown. These Benzidine Derivatives are either breakdown products of benzidine-based substances used as dyes or residuals from use as chemical intermediates in the dyeing process. Notified products include clothing, shoes and textile accessories that may be used by the general population, including products intended for infants, and were originally manufactured in India, China, Turkey and the Philippines (RAPEX 2012).

Table 7-1. Consumer products containing 3,3′-DMB and 3,3′-DMOB notified in RAPEX database from 2010 to September 2012 (RAPEX 2012)

Benzidine Derivative	Consumer products notified in RAPEX	Number of products	Concentration range (mg/kg)	Country of origin
3,3′-DMB	Children’s costume, children’s sweatshirt, jeans, scarves	5	5.16–640	China, India, Philippines
3,3′-DMOB	Baby shoes, children’s cardigan, children’s outfit,	16	8.7–615	China, India, Turkey

	children’s track suit, jeans, shorts, scarves, sleeping bag, socks, sweatshirt for boys			

The EurAzos project (EurAzos 2007) is a European enforcement project conducted in 2007, similar to the RAPEX alert system, which aimed to assess the compliance of textile and leather products in the European market with the provisions regarding the EU22 aromatic amines (EU 2006). There were nine violations reported among 361 textile and leather products analyzed, in which the concentrations of EU22 aromatic amines were found to be above the regulated concentration of 30 mg/kg; two of the nine products contained 3,3′-DMOB (31 mg/kg in a hat and 590 mg/kg in an unidentified product). The countries of origin for these two products were not reported.

A Japanese study surveyed 86 textile products purchased at retail stores in Japan between January and March 2009 for 26 primary aromatic amines released from azo dyes (Kawakami et al. 2010). In addition to the EU22 aromatic amines, four additional aromatic amines (2,4-xylidine, 2,6-xylidine, aniline and 1,4-phenylenediamine) were tested. In total, 117 samples of 86 textile products were analyzed for their content of aromatic amines released from azo dyes when extracted under reductive conditions. Two separate standard European sample processing methods, with slight modifications, were used, depending on the type of material. The EN 14362-1 “without solvent extraction” method (ECS 2003a) was used for 77 samples composed of natural fibres (e.g., cellulosic and protein-based fibres). The EN 14362-2 “with solvent extraction” method (ECS 2003b) was used for 40 samples composed of synthetic fibres (e.g., polyester). Both sample processing methods were conducted for “mixed-fibre samples.” Table 7-4 summarizes the results of the analysis for 3,3′-DMB and 3,3′-DMOB. Based on the results of the analysis, almost all concentrations of the investigated aromatic amines were measured below the 30 mg/kg limit set by the EU. However, 3,3′-DMOB concentrations were found to be particularly high (up to 390 mg/kg) in several cotton placemat samples manufactured in India. However, direct and prolonged exposure from the intended use of placemats is not expected.

Table 7-2. Results of the analysis of 117 samples of 86 commercial textile products purchased in Japan in 2009 for 3,3′-DMB and 3,3′-DMOB (Kawakami et al. 2010)

Benzidine Derivative	LOQ (mg/kg)[a]	Frequency of detects (%) among products analyzed (*n* = 86)	Concentration range by weight in textiles (mg/kg)
3,3′-DMB	0.0125	4.7	0.072–2.4
3,3′-DMOB	0.0175	14.0	0.045–390

Abbreviation: LOQ, limit of quantification

[a] The LOQ given in the study was for the LOQ in the injected solution. The LOQ in the actual product was calculated by multiplying the final sample volume (5 mL) and dividing by the sample weight (1 g).

These three product surveys in Europe and Japan (i.e., EurAzos 2007; Kawakami et al. 2010; RAPEX 2012) indicate that 3,3′-DMB and 3,3′-DMOB can be present in imported products in these foreign markets. Given that the Canadian textile market is composed predominantly of imported products (Industry Canada 2012; Environment Canada and Health Canada 2013b), the potential for these substances to be present in a limited number of imported products in Canada is recognized.

The remaining three Benzidine Derivatives, 3,3′-DMB·2HCl, TODI and 4N-TMB, are not part of the EU22 aromatic amines and therefore were not targeted for analysis in the product surveys mentioned above. These substances are used as intermediates in chemical synthesis, and no consumer products were identified as containing these substances in Canada. The presence of these substances in imported products is not expected except potentially as residuals at low levels; as such, direct exposure of the general population to 3,3′-DMB·2HCl, TODI or 4N-TMB is not expected.

Benzidine-based Substances

Within the Benzidine-based Substances, 26 substances (Table 7-3) are capable of breaking down to one of four EU22 aromatic amines; benzidine, 3,3′-DCB, 3,3′-DMOB or 3,3′-DMB. In the dye industry, there are approximately 250 azo dyes that are based on benzidine (CAS RN 92-87-5), 6 azo dyes that are based on 3,3′-DCB (CAS RN 91-94-1), 95 azo dyes that are based on 3,3′-DMB and 89 azo dyes that are based on 3,3′-DMOB (SCCNFP 2002). These four aromatic amines (benzidine, 3,3′-DCB, 3,3′-DMOB or 3,3′-DMB) were not detected in testing of domestic and imported textile and leather products on the Canadian market (Health Canada 2013) (limits of detection ranged from 1.4 to 1.9 mg/kg; study described above). Notwithstanding the restrictions in other jurisdictions, the presence of some of the EU22 aromatic amines in textile and leather products has been reported in product compliance surveys in other countries (e.g., the RAPEX alert system (RAPEX 2012), the EurAzos project (EurAzos 2007) and the recent Japanese survey (Kawakami et al. 2010)). In these surveys, the detection of an EU22 aromatic amine may suggest the presence of a dye based on the detected aromatic amine; however, the specific dye is not identified. Therefore, while some of these EU22 aromatic amines (benzidine, 3,3′-DCB, 3,3′-DMOB or 3,3′-DMB) may be detected in imported products in compliance and market product surveys in other countries, the available information does not indicate direct and prolonged exposure of the general population of Canada to the 26 Benzidine-based Substances.

Table 7-3. Twenty-six Benzidine-based Substances based on EU22 aromatic amines

Chemical name/acronym	EU22 aromatic amine

Direct Red 28	Benzidine
Direct Brown 95	Benzidine
Direct Blue 8	3,3′-DMOB
Direct Blue 15	3,3′-DMOB
Direct Blue 151	3,3′-DMOB
NAAH·3Li	3,3′-DMOB
BABHS	3,3′-DMOB
NADB·4Li	3,3′-DMOB
NADB·Li·3Na	3,3′-DMOB
NADB·2Li·2Na	3,3′-DMOB
NAAH·Li·2Na	3,3′-DMOB
NAAH·2Li·Na	3,3′-DMOB
NADB·2Li	3,3′-DMOB
NADB·Li·Na	3,3′-DMOB
NADB·3Li·Na	3,3′-DMOB
Acid Red 128	3,3′-DMOB
TCDB	3,3′-DMOB
Direct Blue 14	3,3′-DMB
Direct Red 2	3,3′-DMB
Direct Blue 25	3,3′-DMB
Direct Violet 28	3,3′-DMB
Direct Blue 295	3,3′-DMB
Acid Red 114	3,3′-DMB
Acid Black 209	3,3′-DMB
NAAHD	3,3′-DMB
Direct Red 46	3,3′-DCB

Eight of the Benzidine-based Substances are based on benzidine derivatives that are not EU22 aromatic amines (Table 7-4). No information regarding consumer product use was identified for these Benzidine-based Substances in Canada, except for Acid Red 97. Dermal exposure to Acid Red 97 from dermal contact with textile clothing and leather articles is estimated to range from 2.6×10^{-3} to 4.0×10^{-3} mg/kg body weight (kg-bw) per day for textile clothing and from 2.1×10^{-3} to 7.7×10^{-2} mg/kg-bw for leather articles (refer to Appendix E). Oral exposure due to mouthing of textiles by infants is estimated to be 2.7×10^{-5} mg/kg-bw per day as a conservative estimate. The dermal exposure estimates from textiles are based on conservative assumptions (e.g., full body coverage and direct skin contact). Benzidine-based dyes are relatively water soluble, therefore the effect of laundering is expected to significantly reduce any dye that is not fixed to the textile fibre, thereby reducing exposures over time. It is not expected that Acid Red 97 would be present in all consumer products made of textiles in Canada. Therefore, exposures were derived assuming, based on professional judgement, that there is a 10% probability that this substance is used in dyeing products made of textile in Canada. From the limited data available (Danish EPA 1998; Brüschweiler et al. 2014), the detection of most non-EU22 amines in textiles is usually less than 10%. Accordingly, the presence of associated dyes in textiles would be the same or lower. This adjustment factor of 10% used in this assessment is similar to the 8% value used in the Danish assessment in estimating exposures to aromatic amines and azo dyes from textile garments in the Dutch market (Zeilmaker et al. 1999). See Appendix E for further explanation on the estimated exposures to Acid Red 97.

As the non-EU22 benzidine derivatives were not within the scope of the three product surveys described above (i.e., EurAzos 2007; Kawakami et al. 2010; RAPEX 2012), no information is available from these surveys on the presence of these benzidine derivatives in products in Europe or Japan. From the limited data available (Danish EPA 1998; Brüschweiler et al. 2014), the detection of most non-EU22 amines in textiles is usually less than 10%. Accordingly, the presence of associated dyes in textiles would be the same or lower. Testing of products on the Canadian market included the analysis of 2,2′-DCB and 2,2′-DMB in imported and domestic products, and did not detect these two benzidine derivatives (limits of detections of 3.1 ppm for 2,2′-DMB and 1.0 ppm for 2,2′-DCB; Health Canada 2013). Overall, direct and prolonged exposure to the 7 remaining Benzidine-based Substances from contact with textiles and leather is not expected.

Table 7-4. Eight Benzidine-based Substances based on non-EU22 benzidine derivatives

Chemical name/acronym	non-EU22 benzidine derivatives
Acid Red 97	2,2′-DSB
NAADD	2,2′-DSB
Acid Orange 56	2,2′-DSB
BAHSD	2,2′-DCB
BDAAH	3,3′-DCAB
Direct Blue 158	3,3′-DCMB
Acid Red 99	2,2′-DMB
BADB	2,2′-DMB

Abbreviations: 2,2′-DCB, 2,2′-dichlorobenzidine; 2,2′-DMB, 2,2′-dimethylbenzidine; 2,2′-DSB, 2,2′-disulfobenzidine; 3,3′-DCAB, 3,3′-dicarboxybenzidine; 3,3′-DCMB, 3,3′-di(carboxymethoxy)benzidine

The two Benzidine-based Cationic Indicators (TDBD and TDBPD) were identified for use primarily as laboratory reagents (Merck Index 2001; Sigma-Aldrich Canada 2010; Ullmann's Encyclopedia 2010), and the Benzidine-based Precursor Naphthol AS-BR was identified as a dye precursor (Freeman 2011). Use of these three substances by the general population is not expected; therefore, exposure to these three substances is not expected.

7.2 Health Effects Assessment

Carcinogenicity and genotoxicity are the critical health effects of potential concern for Aromatic Azo and Benzidine-based Substances (Environment Canada and Health Canada 2013). The mechanism by which Benzidine-based Substances exert their toxicity involves the reductive cleavage of the azo bonds and the subsequent release of the free aromatic amines. These aromatic amines are, in turn, converted to reactive electrophilic intermediates through metabolic oxidation (Environment Canada and Health Canada 2013).

The health effects of the Benzidine-based Substances are evaluated in this assessment by examining their ability to undergo reductive cleavage and their hazard potential. This analysis is based on consideration of the available information and presented in the next two sections. Similarly, the hazard potential of the five Benzidine Derivatives is evaluated and discussed. Health effects data on two substances previously assessed in the Challenge Initiative (Acid Red 111 and Direct Black 38) were included to inform the health effects assessment of the Benzidine-based Substances.

The focus of the health effects assessment was on those Benzidine-based Substances and Benzidine Derivatives for which exposure to the general population is expected (see section Exposure Assessment).

7.2.1 Azo Bond Cleavage Potential

The azo bond cleavage potential of the substances considered in this assessment was determined based on several lines of evidence that have been previously discussed (Environment Canada and Health Canada 2013). The types of information considered in this assessment range from *in vivo* assays to read-across approaches.

In vivo metabolism studies provide the most direct evidence for reductive cleavage, and this type of study was found for 10 Benzidine-based Dyes (Table 7-5). In all instances, one or more of the released aromatic amines were identified in the urine and feces of one or more mammalian species that were orally exposed to the dye. The amounts of the aromatic amines present in the urine and feces were greater than those present as impurities in the testing material, indicating *in vivo* cleavage of the azo bond (Rinde 1974; Rinde and Troll 1975; Lynn et al. 1980; Nony and Bowman 1980; Robens et al. 1980; Bowman et al. 1982, 1983; Kennelly et al. 1982; Levine et al. 1982; Nony et al. 1983; NTP 1983). Reactive aromatic amine metabolites were also found when hemoglobin adducts were used to monitor the bioavailability of Direct Red 28 and Direct Red 46 (Birner et al. 1990; Sagelsdorff et al. 1996).

In vitro metabolism studies were found for six Benzidine-based Dyes (Table 7-5). The aromatic amines generated from the reductive cleavage of the azo bond were identified following incubation of the dye with either intestinal contents from various species or human skin cultures. In all these studies, the potential for reductive cleavage was demonstrated (Hartman et al. 1978; Cerniglia et al. 1982a, b, 1986; Bos et al. 1986a; Chung et al. 1992; Platzek et al. 1999). The results of studies investigating liver metabolism, in contrast, were mixed (Martin and Kennelly 1981; Bos et al. 1984). Only Direct Black 38 and Direct Brown 95 released their aromatic amine or its acetylated form following incubation with rat liver supernatant or rat hepatocytes. In general, liver metabolism plays a minor or negligible role in the azo reduction of dyes derived from benzidine or its derivatives (Martin and Kennelly 1981).

Another line of evidence that was considered in the assessment of reductive cleavage potential is the results obtained from the Ames assay under reductive conditions. These reductive conditions include incubation with intestinal contents, incorporation of the Prival modifications or presence of sodium dithionite. If the Ames test yielded positive results only after such conditions were employed, then the potential for the substance to cleave into genotoxic metabolites *in vivo* was inferred (Environment Canada and Health Canada 2013). Eleven Benzidine-based Dyes were evaluated in this type of assay, and all had data to support azo bond cleavage (Table 7-5).

In the absence of empirical data, the reductive cleavage potential of a Benzidine-based Substance can be inferred based on read-across among closely related analogues (Environment Canada and Health Canada 2013). These closely related analogues were determined based on a number of factors, including the number of azo bonds, the number of rings, the types of rings and water solubility. Substances with similar properties were assumed to be similarly likely to undergo azo reductive cleavage. The division of Benzidine-based Substances into structurally related groups (Table 7-5) allowed for read-across from substances with empirical data for azo bond reductive cleavage to those with no data. For two structurally related groups and a number of stand-alone substances, however, there were no empirical data that could be used to infer azo bond reductive cleavage. In such instances, a general read-across was done for Benzidine-based Substances, based on the fact that for this class of substances, all available empirical data indicate that reductive cleavage occurs. Therefore, the genotoxicity and carcinogenicity of the released aromatic amines are also considered in the assessment of the Benzidine-based Substances.

Table 7-5. Information considered in the determination of azo bond reductive cleavage

Subgroup[a]	**Chemical Name/ acryonym**	**ADME data**	***In vitro* metabolism data**	**Ames assay (positive only with reductive conditions)**	**Read-across**
Acid Dyes	Acid Red 114	X			
Acid Dyes	Acid Red 111[b]			X	

Subgroup[a]	**Chemical Name/ acryonym**	**ADME data**	***In vitro* metabolism data**	**Ames assay (positive only with reductive conditions)**	**Read-across**
Acid Dyes	Acid Red 128				X
Acid Dyes	Acid Red 99			X	
Acid Dyes	Acid Red 97				X
Acid Dyes	Acid Black 209				X
Acid Dyes	NAAHD				X
Acid Dyes	NAADD				X
Acid Dyes	BADB				X
Acid Dyes	Acid Orange 56				X
Direct Dyes	Direct Brown 95	X	X	X	
Direct Dyes	Direct Blue 14	X	X	X	
Direct Dyes	Direct Blue 295				X
Direct Dyes	Direct Red 2	X	X	X	
Direct Dyes	Direct Blue 25	X		X	
Direct Dyes	Direct Violet 28				X
Direct Dyes	Direct Red 28	X	X	X	
Direct Dyes	Direct Red 46	X		X	
Direct Dyes	Direct Blue 158				X
Direct Dyes	Direct Blue 15	X	X	X	
Direct Dyes	NADB·4Li				X
Direct Dyes	NADB·Li·3Na				X
Direct Dyes	NADB·2Li·2Na				X
Direct Dyes	NADB·3Li·Na				X
Direct Dyes	Direct Black 38[b]	X	X		
Direct Dyes	Direct Blue 8	X		X	
Direct Dyes	Direct Blue 151				X
Direct Dyes	NADB·2Li				X
Direct Dyes	NADB·Li·Na				X
Direct Dyes	BABHS			X	
Direct Dyes	NAAH·3Li				X
Direct Dyes	NAAH·Li·2Na				X
Direct Dyes	NAAH·2Li·Na				X
Direct Dyes	BAHSD				X
Direct Dyes	BDAAH				X
Precursors	TCBD				X

Abbreviations: ADME, absorption, distribution, metabolism, elimination

[a] Acid Dyes, Direct Dyes and Precursors refer to the Benzidine-based Acid Dyes, Benzidine-based Direct Dyes and Benzidine-based Precursors subgroups, respectively. The substances are further divided into structurally related groups (Environment Canada and Health Canada 2013). These structurally related groups are delineated by

double lines. An "X" is placed where empirical data are available or to indicate the type of read-across that was done to determine azo bond reductive cleavage for the dye. The Benzidine-based Precursor Naphthol AS-BR and the two Benzidine-based Cationic Indicators TDBPD and TDBD do not have an azo bond and are therefore excluded from this table.

[b] Data on Acid Red 111 and Direct Black 38 are included to inform the health effects assessment.

The information available to determine azo bond reductive cleavage for this class of substances is generally strong. The reductive cleavage potential for many substances and structurally related groups is based on several types of evidence. All the Benzidine-based Acid and Direct Dyes evaluated in this assessment as well as the Benzidine-based Precursor TCDB are expected to release aromatic amines. Confidence in the read-across approach for substances without empirical data is high, as they have been grouped together based on their similar uses, properties and structures. The carcinogenicity and genotoxicity of the released aromatic amines are therefore considered when determining the hazard potential of Benzidine-based Acid and Direct Dyes as well as the Benzidine-based Precursor TCDB.

In contrast, the two Benzidine-based Cationic Indicators, TDBPD and TDBD, and the Benzidine-based Precursor Naphthol AS-BR do not have the typical azo bond. Therefore, only the hazard potential of these substances is considered in the following section.

7.2.2 Health Effects

Carcinogenicity and genotoxicity are the critical health effects of potential concern for Aromatic Azo and Benzidine-based Substances and, in particular, Benzidine-based Dyes. Studies for these endpoints, especially for carcinogenicity, are not available for many of these dyes. To address the lack of data for benzidine- and benzidine congener–derived dyes, the Benzidine Dye Initiative was established by the US National Toxicology Program (NTP). This research program generated data for two benzidine derivatives and a select group of prototypical dyes derived from those amines and applied the basic information generated from those studies to the toxicity and carcinogenicity associated with other benzidine- and benzidine derivative–based dyes after conducting only a small number of experiments (Morgan et al. 1994). This potential for read-across is also considered, as applicable.

When available, empirical data from carcinogenicity and genotoxicity studies for the Benzidine-based Substances that are being assessed, as well as for relevant aromatic amine metabolites, were considered (see Tables 18 and 19). When required, read-across from substances that release similar aromatic amine metabolites, SAR analyses and (Q)SAR models were also used.

The structures of the potential metabolites released for each Benzidine-based Substance were proposed based on theoretical cleavage of the azo bond. The resulting structures were used to identify CAS RNs associated with each metabolite, when possible.

Benzidine-based Substances That May Release Benzidine, 3,3′-DMOB, 3,3′-DMB or 3,3′-DCB

Of the Benzidine-based Substances included in this assessment, 26 may release benzidine or one of three benzidine derivatives that have been previously assessed and classified by international agencies. Benzidine, 3,3′-DMOB, 3,3′-DMB and 3,3′-DCB are groups 1 and 2B International Agency for Research on Cancer (IARC) carcinogens (known and possible human carcinogens, respectively). They are also regulated in Europe as part of the EU22 aromatic amines. Benzidine and these benzidine derivatives are responsible for the high hazard potential of the Benzidine-based Substances. Studies with reproductive and developmental endpoints are available for seven of these dyes (Table 7-6).

Table 7-6. Available information for genotoxicity and carcinogenicity of Benzidine-based Substances that may release benzidine, 3,3′-DMOB, 3,3′-DMB or 3,3′-DCB

Chemical name/acronym	Subgroup[a]	Genotoxicity/ carcinogenicity (empirical data)	Benzidine Derivative released
Direct Red 28 [b]	Direct Dyes	Positive/n.d.	Benzidine
Direct Brown 95 [b]	Direct Dyes	Positive/positive	Benzidine
Direct Blue 8	Direct Dyes	Positive/n.d.	3,3′-DMOB
Direct Blue 15 [b]	Direct Dyes	Positive/positive	3,3′-DMOB
Direct Blue 151	Direct Dyes	Positive/n.d.	3,3′-DMOB
Acid Red 128	Acid Dyes	n.d./n.d.	3,3′-DMOB
NAAH·3Li	Direct Dyes	n.d./n.d.	3,3′-DMOB
BABHS	Direct Dyes	Positive/n.d.	3,3′-DMOB
NADB·4Li	Direct Dyes	n.d./n.d.	3,3′-DMOB
NADB·2Li·2Na	Direct Dyes	n.d./n.d.	3,3′-DMOB
NADB·Li·3Na	Direct Dyes	n.d./n.d.	3,3′-DMOB
NADB·Li·Na	Direct Dyes	n.d./n.d.	3,3′-DMOB
NADB·2Li	Direct Dyes	n.d./n.d.	3,3′-DMOB
NAAH·2Li·Na	Direct Dyes	n.d./n.d.	3,3′-DMOB
NAAH·Li·2Na	Direct Dyes	n.d./n.d.	3,3′-DMOB
NADB·3Li·Na	Direct Dyes	n.d./n.d.	3,3′-DMOB
TCDB	Precursors	n.d./n.d.	3,3′-DMOB
Direct Blue 14 [b]	Direct Dyes	Positive/positive	3,3′-DMB
Direct Red 2 [b]	Direct Dyes	Positive/n.d.	3,3′-DMB

Chemical name/acronym	Subgroup[a]	Genotoxicity/ carcinogenicity (empirical data)	Benzidine Derivative released
Direct Blue 25	Direct Dyes	Positive/n.d.	3,3′-DMB
Acid Red 114	Acid Dyes	Positive/positive	3,3′-DMB
Direct Blue 295	Direct Dyes	n.d./n.d.	3,3′-DMB
Direct Violet 28 [b]	Direct Dyes	n.d./n.d.	3,3′-DMB
Acid Black 209	Acid Dyes	n.d./n.d.	3,3′-DMB
NAAHD	Acid Dyes	n.d./n.d.	3,3′-DMB
Direct Red 46	Direct Dyes	Positive/n.d.	3,3′-DCB

Abbreviations: n.d., no data

[a] Acid Dyes, Direct Dyes and Precursors refer to the Benzidine-based Acid Dyes, Benzidine-based Direct Dyes and Benzidine-based Precursors subgroups, respectively.

[b] Studies with reproductive and developmental endpoints are available for these dyes.

[c] The dihydrochloride salt of 3,3′-DMB is also covered in this assessment.

Benzidine-based Substances That May Release Benzidine

Direct Red 28 and Direct Brown 95 may release benzidine following azo bond reductive cleavage (refer to Table 7-6). In addition, data on Direct Black 38 were included to inform the health effects assessment. Both *in vitro* and *in vivo* genotoxicity assays are essentially positive for all three dyes. *In vitro* mutagenicity studies indicate that reductive metabolism and metabolic activation are required for Direct Red 28 and Direct Brown 95 to exert mutagenicity, while Direct Black 38 requires only metabolic activation (Gregory et al. 1981; Martin and Kennelly 1981; Prival and Mitchell 1982; Robertson et al. 1982; Brown and Dietrich 1983; Reid et al. 1983, 1984; Joachim and Decad 1984; Prival et al. 1984; Joachim et al. 1985; Cerniglia et al. 1986; De France et al. 1986; Krishna et al. 1986; Mortelmans et al. 1986; Chung et al. 2006; ILS 2011a). Direct Red 28 was also weakly positive for DNA repair induction in hamster hepatocytes and positive for DNA damage in mammalian cells (Kornbrust and Barfknecht 1984; Bafana et al. 2009b). Direct Brown 95, however, was negative for chromosomal aberrations, unscheduled DNA synthesis and sister chromatid exchange in mammalian cells (Joachim and Decad 1984; Galloway et al. 1987); this was presumably due to the absence of reductive conditions. *In vivo* genotoxicity assays were positive for DNA damage for Direct Red 28 (Kennelly et al. 1984a; Yi et al. 1993), for mutagenicity and unscheduled DNA synthesis for Direct Black 38 and Direct Brown 95 (Nony 1979; Bos et al. 1984, 1986a, b; Joachim and Decad 1984; Joachim et al. 1985; Beije 1987; Ashby and Mohammed 1988), as well as for chromosome damage and DNA damage for Direct Black 38 (Beije 1987; Tsuda et al. 2000).

A number of studies showing a carcinogenic effect in laboratory animals were identified for Direct Black 38 and Direct Brown 95. Hepatocellular carcinomas and mammary carcinomas were found in mice orally exposed to Direct Black 38 in their drinking water for 55–60 weeks (US EPA 1987a). In rats, Direct Black 38 produced hepatocellular carcinomas within 13 weeks after it was administered in the diet and carcinomas in the urinary bladder, liver and colon after it was administered in drinking water (Okajima et al. 1975; Robens et al. 1980). In a well-conducted 13-week study, Direct Brown 95 produced neoplastic nodules in the livers of four of eight female rats, one of which showed a hepatocellular carcinoma (US EPA 1987b). Due to the increased mortality in male rats, neoplastic lesions were not seen, but significant increases in basophilic foci were observed in these rats.

IARC has evaluated both Direct Black 38 and Direct Brown 95 and previously classified both as group 2A (probable human carcinogens). The working group found that while there is *inadequate evidence* in humans for the carcinogenicity of dyes metabolized to benzidine, there is *sufficient evidence* in experimental animals for the carcinogenicity of both dyes (IARC 2010a). More recently, IARC has classified dyes that are metabolized to benzidine as group 1 (carcinogenic to humans) (IARC 2012a). The conclusion was based on a) *sufficient evidence* in experimental animals for the carcinogenicity of dyes metabolized to benzidine; b) *strong mechanistic evidence* indicating that benzidine-based dyes are converted by azo reduction to benzidine in humans and in experimental animals and, consequently, produce DNA adducts and genotoxic effects similar to those of benzidine; c) *sufficient evidence* in humans and in experimental animals for the carcinogenicity of benzidine; and d) *evidence* that benzidine-based dyes induce chromosomal aberrations in humans and in all experimental animal species studied. In the EU, benzidine-based azo dyes are classified as carcinogenicity category 1B (known to be carcinogenic to humans) with hazard code H350 (“may cause cancer”) (ESIS ©1995–2012). Dyes metabolized to benzidine are listed as “known to be human carcinogens” in the US NTP *Report on Carcinogens* (NTP 2011).

The Government of Canada has also evaluated Direct Black 38 and has recognized that it may be harmful to human health (Environment Canada and Health Canada 2009).

The Government of Canada has evaluated benzidine and concluded that it was harmful to human health, based on genotoxicity and carcinogenicity (Canada 1993a). More recently, IARC concluded that studies on occupational exposure to benzidine and incidences of bladder cancer showed “consistent positive associations with some indication of dose–response relationships.” In addition, oral, subcutaneous and intraperitoneal administration of benzidine to mice, rats, dogs and hamsters resulted in tumours at a variety of sites. On this basis, benzidine was classified as a group 1 carcinogen (carcinogenic to humans) by IARC (2010a, 2012b).

The health effects of Direct Red 28 and Direct Brown 95 are expected to be similar to those of benzidine, due to the release of benzidine following azo bond reductive cleavage.

3,3′-DMOB and Benzidine-based Substances That May Release 3,3′-DMOB

Fifteen of the Benzidine-based Substances may release 3,3′-DMOB upon azo bond reductive cleavage (refer to Table 18).

In vitro genotoxicity studies were found for Direct Blue 8, Direct Blue 15, Direct Blue 151 and BABHS. All four dyes were mutagenic in the presence of metabolic activation and reductive conditions in bacterial mutagenicity assays (EI DuPont de Nemours and Co. Inc. 1978; Gregory et al. 1981; Brown and Dietrich 1983; Prival et al. 1984; Reid et al. 1984; Krishna et al. 1986; Mortelmans et al. 1986; Zhou et al. 1987; ILS 2011b). While Direct Blue 15 and BABHS also showed some equivocal or positive results under metabolic activation only, incorporation of reductive conditions resulted in an increase in mutagenic potency for Direct Blue 15 (Prival et al. 1984; Reid et al. 1984). Incubation of Direct Blue 15 with mammalian cells did not produce increases in sister chromatid exchanges or chromosomal aberrations with or without metabolic activation, but the assays were not carried out under reductive conditions (Galloway et al. 1987).

An *in vivo* genotoxicity study and a carcinogenicity study were identified for Direct Blue 15. Direct Blue 15 produced DNA damage in the liver of male mice 3 hours after treatment (Tsuda et al. 2000). When rats were exposed to Direct Blue 15 (purity 50%; 35 impurities, including 3,3′-DMOB·2HCl [836–1310 ppm] and benzidine [< 1 ppm]) in their drinking water for 22 months (early termination due to extensive mortality associated with chemical-related neoplasia), neoplasms were observed as early as 9 months in the Zymbal gland and clitoral gland. At 22 months, neoplasms were found at multiple sites, including Zymbal gland, skin, oral cavity and preputial or clitoral gland, in a dose-dependent manner. Neoplasms were also observed in the small and large intestine, liver, uterus and brain. The incidence of mononuclear cell leukemia was also increased in female rats (NTP 1992). Given the dye's low purity, there is uncertainty as to whether the effects observed in this otherwise well-conducted NTP study can be solely attributed to Direct Blue 15. It was, however, the only study identified for dyes based on 3,3′-DMOB.

IARC has evaluated Direct Blue 15 and has classified it as a group 2B carcinogen (possibly carcinogenic to humans). The working group has found that while there is *inadequate evidence* in humans, there is *sufficient evidence* in experimental animals for the carcinogenicity for Direct Blue 15 (IARC 1993a).

3,3′-DMOB was classified as a group 2B carcinogen (possibly carcinogenic to humans) by IARC; the working group concluded that "there is *sufficient evidence* in experimental animals for the carcinogenicity of 3,3′-dimethoxybenzidine" (IARC 1974a, 1987a, 2010b). In the EU, 3,3′-DMOB and 3,3′-DMOB-based azo dyes are classified as carcinogenicity category 1B (known to be carcinogenic to humans) with hazard code H350 ("may cause cancer") (ESIS ©1995–2012). 3,3′-DMOB and dyes metabolized to

3,3′-DMOB are listed as “reasonably anticipated to be human carcinogens” in the US NTP *Report on Carcinogens* (NTP 1998, 2011). Data on the hydrochloride salts of 3,3′-DMOB were considered relevant, as these salts are expected to dissociate in physiological media to generate 3,3′-DMOB and are therefore considered to be toxicologically equivalent.

Following administration of 3,3′-DMOB·2HCl in the drinking water for up to 21 months, tumours were induced in rats at multiple sites, including the skin, Zymbal gland and intestines in both sexes, as well as in the liver, preputial gland and oral cavity in males and in the clitoral and mammary glands in females (Morgan et al. 1990; NTP 1990). The study was originally intended to have a 2-year treatment period, but it was terminated at 21 months because of reduced animal survival in all dose groups, primarily due to neoplasm-related deaths. At an interim (9 months) sacrifice of some high-dose animals, malignant and benign tumours were observed at multiple sites, indicating an early onset of some treatment-related tumours. At the 21-month time point, tumour incidences were clearly increased and showed a dose–response relationship.

Rats given 3,3′-DMOB by stomach intubation for 12–13 months developed tumours in the bladder, intestine, skin, ovaries, mammary gland and Zymbal gland (Pliss 1963, 1965; Hadidian et al. 1968). In a study in which male and female hamsters were given 3,3′-DMOB in the diet for 144 weeks (approximately 2.7 years), there was a significant increase in the incidence of forestomach papillomas, and one treated animal developed a rare transitional cell carcinoma in the bladder (Saffiotti et al. 1967; Sellakumar et al. 1969). No evidence of carcinogenicity was observed in a 2-year drinking water study in mice (Schieferstein et al. 1990).

No studies on the relationship between exposure to 3,3′-DMOB alone and cancer in humans were identified. In the epidemiological studies identified, subjects were exposed to benzidine or other carcinogenic aromatic amines in addition to 3,3′-DMOB, and therefore the studies cannot be used to evaluate the effects of 3,3′-DMOB exposure specifically. IARC has concluded that the evidence for carcinogenicity in humans is insufficient (IARC 2010b).

Benchmark doses (BMD) associated with a 10% increase in tumour incidence above controls (i.e., the BMD_{10}) and the corresponding lower limit of a one-sided 95% confidence interval ($BMDL_{10}$) were derived for 3,3′-DMOB·2HCl using the US EPA Benchmark Dose Software (BMDS version 2.3.1) (US EPA 2013). The BMD approach, which includes dose–response modelling, provides a quantitative alternative to the traditional dose–response assessment. BMD_{10} and $BMDL_{10}$ values were calculated for nine tumour sites from the 21-month rat drinking water study (NTP 1990) (see Appendix F for details). The lowest calculated BMD_{10} for 3,3′-DMOB·2HCl is 0.32 mg/kg-bw per day, based on 3,3′-DMOB·2HCl-induced skin basal cell or sebaceous gland neoplasms in male F344/N rats; the lower 95% confidence limit ($BMDL_{10}$) for this value is 0.22

mg/kg-bw per day. BMD_{10} and $BMDL_{10}$ values of 0.91 and 0.66 mg/kg-bw per day, respectively, were obtained for female clitoral gland neoplasms, which supports the high tumour-inducing potency of 3,3′-DMOB in rats. When the lowest $BMDL_{10}$ for 3,3′-DMOB·2HCl (0.22 mg/kg-bw per day) is adjusted for the molecular weight difference, it is equivalent to a $BMDL_{10}$ of 0.17 mg/kg-bw per day for 3,3′-DMOB. The derived $BMDL_{10}$ values are similar in magnitude to the previously published LTD_{10} (the lowest tumorigenic dose associated with a 10% increase in tumour incidence above controls), which is 0.12 mg/kg-bw per day for 3,3′-DMOB·2HCl (CPDB 2012).

The majority of *in vitro* and *in vivo* genetic toxicology test results for 3,3′-DMOB were positive. *In vivo*, 3,3′-DMOB induced chromosomal aberrations and sister chromatid exchanges in the bone marrow of mice treated by injection (Gorecka-Turska 1983; You et al. 1993). However, a micronucleus assay was negative in the bone marrow of mice treated by intraperitoneal injection and gave mixed results when the mice were dosed by gavage (Morita et al. 1997). DNA damage was detected by the comet assay in tissues of mice following oral gavage (Sasaki et al. 1999; Martelli et al. 2000); tests for sex-linked recessive lethal mutations in *Drosophila melanogaster* adults and larvae were negative (Yoon et al. 1985; Zimmering et al. 1989). In mammalian cells *in vitro*, 3,3′-DMOB was mutagenic in the mouse lymphoma assay (Mitchell et al. 1988; Myhr and Caspary 1988) and induced DNA and chromosome damage (Martin et al. 1978; Probst et al. 1981; Galloway et al. 1985; JETOC 2000; Martelli et al. 2000; Chen et al. 2003).In reverse mutation assays in bacteria (Ames test), 3,3′-DMOB was generally positive in *Salmonella typhimurium* strains TA98, TA100, TA102 and TA1538 with metabolic activation, but negative without metabolic activation. Negative results were obtained in TA1535 and TA1537 both with and without metabolic activation (Probst et al. 1981; Haworth et al. 1983; Krishna et al. 1986; You et al. 1993; Chung et al. 2000; Makena and Chung 2007). Other bacterial tests (SOS/umu, forward mutation) were negative both with and without metabolic activation (Nakamura et al. 1987; Von der Hude et al. 1988; Shimada et al. 1989).

The health effects of the 15 3,3′-DMOB-based substances are expected to be similar to those of 3,3′-DMOB, due to the release of 3,3′-DMOB following azo bond reductive cleavage.

3,3′-DMB and Benzidine-based Substances That May Release 3,3′-DMB

Eight of the Benzidine-based Substances may release 3,3′-DMB following azo bond reductive cleavage (refer to Table 18).

In vitro genotoxicity studies were found for Direct Blue 14 (also referred to as trypan blue), Direct Blue 25, Direct Red 2 and Acid Red 114. All four dyes were mutagenic in the presence of reductive metabolism followed by oxidative metabolism with S9 liver enzymes (Brown et al. 1978; Hartman et al. 1978; Elliott and Gregory 1980; Gregory et al. 1981; Prival and Mitchell 1982; Brown and Dietrich 1983; Prival et al. 1984; Reid et al. 1984; Joachim et al. 1985; De France et al. 1986; Krishna et al. 1986; Mortelmans et al. 1986; Cameron et al. 1987; Zeiger et al. 1987; Dellarco and Prival 1989; NTP 1991a; Chung et al. 2006). Acid Red 114 was also mutagenic without prior reduction; positive results were observed in the presence of metabolic activation for *Salmonella typhimurium* strains TA98 and TA1538 (ETAD 1983a, 1985, 1986; Mortelmans et al. 1986; NTP 1991a). Direct Red 2 exhibited mutagenicity in the bacterial forward mutation assay in the presence of metabolic activation; this is in contrast to its results in the Ames assay (Prival and Mitchell 1982). In mammalian cells, Direct Red 2 did not induce any DNA repair (Von der Hude et al. 1988), nor did Acid Red 114 induce sister chromatid exchange or chromosomal aberrations in Chinese hamster ovary cells with or without S9 activation (NTP 1991a). Reductive metabolism was, however, not used in these cytogenetic tests. Direct Blue 14 was positive for chromosomal aberrations, but negative for mouse lymphoma, and mixed results were obtained for induction of DNA repair and morphological transformation (Joneja and Ungthavorn 1968; Amacher and Zelljadt 1983; Joachim and Decad 1984; Kornbrust and Barfknecht 1984, 1985; Longstaff et al. 1984; Cameron et al. 1987; Von der Hude et al. 1988). Addition of 2 mM flavin mononucleotide (FMN) had no effect on induction of DNA repair, and no other assays conducted in mammalian cells were performed under reductive conditions.

Among the *in vivo* genotoxicity assays identified for these Benzidine-based Substances, a dose-dependent increase in chromosomal damage was observed in male mice exposed to Direct Red 2 by oral gavage. When mice were treated with both Direct Red 2 and acidified water, which reduces indigenous intestinal microflora, a significant decrease in micronucleus formation was observed (Rajaguru et al. 1999). Hepatocytes of rats orally exposed to purified Direct Blue 14 displayed unscheduled DNA synthesis (Joachim and Decad 1984). In addition, urine extracts of rats treated with Direct Blue 14 at 500 mg/kg-bw were weakly mutagenic in TA1538 with metabolic activation (Joachim et al. 1985). Chromosomal aberrations were also found in normal-looking embryos from pregnant mice treated on gestational day 9 with Direct Blue 14 at 500 mg/kg-bw (Joneja and Ungthavorn 1968). DNA repair and DNA damage assays, however, were negative for Direct Blue 14 (Kornbrust and Barfknecht 1985; Tsuda et al. 2000). Similarly, no increase in sex-linked recessive lethal mutations in *Drosophilia melanogaster* and no DNA damage in mouse tissues were observed following exposure to Acid Red 114 (Woodruff et al. 1985; Tsuda et al. 2000).

Carcinogenicity studies were identified for Direct Blue 14 and Acid Red 114. Numerous studies using various suppliers of Direct Blue 14 and different routes of exposure have investigated the carcinogenicity of Direct Blue 14. Many of these studies were reviewed by IARC (1975). Direct Blue 14 produced reticulum cell sarcomas, mainly of the liver, as well as fibrosarcomas at the site of injection in rats following subcutaneous or intraperitoneal injection (Gillman and Gillman 1952; Simpson 1952; Brown and Thorson 1956; Brown 1963a, b; Papacharalampous 1966; Gillman et al. 1973; Field et al. 1977;

Ford and Becker 1982). Tumours were found in other organs only when the liver was involved (Brown and Thorson 1956), indicating that metabolism of the dye was required for carcinogenicity. Liver spindle cell sarcomas were also induced in rats by single intraperitoneal injections (Papacharalampous 1957). In a 40-week study in which rats were exposed to four forms of Direct Blue 14 (Grubler, crude synthesized, dialysed and pure), a decrease in potency was observed as more purified forms of the dye were used. Only stage 1 or at most stage 2 changes in the liver were observed when the rats were treated with the purified dye (Field et al. 1977). Experiments conducted in rats that were orally exposed to Direct Blue 14 or in mice exposed by subcutaneous injection were not evaluated by IARC because of the small number of animals used or because the adequacy of the dose used could not be assessed. In a more recent study, neoplasms were observed in the liver and occasionally in the portahepatic lymph nodes of female mice subcutaneously exposed to Direct Blue 14 for 52 weeks. Similar types of tumours were also seen following a 6-week treatment and a latency period of 20 months (Ford and Becker 1982).

Acid Red 114 was tested for carcinogenicity in a 2-year drinking-water study. A clear carcinogenic response was produced in the skin, Zymbal gland and liver of male and female rats and in the clitoral gland, oral cavity epithelium, small and large intestine, and lung in female rats after 2 years. Treatment-related increases in the incidences of neoplasms were also seen in the oral cavity epithelium, adrenal gland and lungs of male rats and in mammary glands and adrenal glands in female rats. An increase in mononuclear cell leukemia was observed in female rats. Neoplasms were found at various sites at the interim time points of 9 and 15 months, and the number of neoplasms at these sites increased with time (NTP 1991a). The malignancy of moderately well and well differentiated preputial gland carcinomas, malignant epidermal basal cell tumours and epidermal squamous cell carcinomas from this 2-year study was confirmed in transplantation studies (Ulland et al. 1989).

IARC has reviewed Direct Blue 14 and Acid Red 114 and has classified each of them as a group 2B carcinogen (possibly carcinogenic to humans) (IARC 1987b, 1993b, 1997). 3,3′-DMB is also classified by IARC as a group 2B carcinogen (possibly carcinogenic to humans) (IARC 1972, 1987c, 2010d). 3,3′-DMB and 3,3’-DMB-based dyes are also classified as carcinogenicity category 1B in the EU, with hazard code H350 (“may cause cancer”) (ESIS ©1995–2012). 3,3′-DMB and dyes known to metabolize to 3,3′-DMB are listed as “reasonably anticipated to be human carcinogens” by the US NTP (NTP 2011). Data on the hydrochloride salts of 3,3′-DMB were considered relevant, as these salts are expected to dissociate in physiological media to generate 3,3′-DMB and are therefore considered to be toxicologically equivalent. The dihydrochloride salt of 3,3′-DMB is also specifically addressed in this assessment.

The oral administration of 3,3′-DMB·2HCl in drinking water to rats for 14 months induced tumours at multiple sites in both sexes, including the skin, Zymbal gland, liver, oral cavity and gastrointestinal tract, as well as in the lung and preputial gland in males and in the mammary and clitoral glands in females (Morgan et al. 1991; NTP 1991b). This study was originally intended to have a 2-year treatment period, but it was terminated at 14 months because of reduced animal survival in all dose groups, due primarily to neoplasm-related deaths. At an interim (9 months) sacrifice of some high-dose animals, malignant and benign tumours were observed at multiple sites, indicating an early onset of some treatment-related tumours. At the 14-month time point, tumour incidences were clearly increased and showed a dose–response relationship. In an earlier study, 3,3′-DMB given to female rats by gavage induced mammary tumours (Griswold et al. 1968).

In mice given 3,3′-DMB·2HCl in drinking water for up to 116 weeks, there was a dose-related increase in the incidence of lung neoplasms, but only in males that died or were sacrificed moribund before study termination (Schieferstein et al. 1989). Zymbal gland tumours were observed in two studies in which rats were administered 3,3′-DMB by subcutaneous injection (Spitz et al. 1950; Pliss and Zabezhinsky 1970). A carcinogenic effect was not observed in hamsters following oral administration of 3,3′-DMB (Saffiotti et al. 1967). No case reports or epidemiological studies on 3,3′-DMB were identified.

BMD_{10} and the corresponding $BMDL_{10}$ values were derived for 3,3′-DMB·2HCl using the US EPA Benchmark Dose Software (BMDS version 2.3.1) (US EPA 2013). BMD_{10} and $BMDL_{10}$ values were calculated for 11 tumour sites from the 14-month rat drinking water study (NTP 1991b) (see Appendix G for details). The lowest calculated $BMDL_{10}$ for 3,3′-DMB·2HCl is 0.51 mg/kg-bw per day, derived from one of the lowest calculated BMD_{10} values of 1.07 mg/kg-bw per day, based on 3,3′-DMB·2HCl-induced skin basal cell neoplasms in male F344/N rats. In addition, $BMDL_{10}$ and BMD_{10} values of 0.59 and 0.76 mg/kg-bw per day, respectively, were obtained for female clitoral gland neoplasms, which supports the high tumour-inducing potency of 3,3′-DMB in rats. When the lowest $BMDL_{10}$ for 3,3′-DMB·2HCl (0.51 mg/kg-bw per day) is adjusted for the molecular weight difference, it is equivalent to a $BMDL_{10}$ of 0.38 mg/kg-bw per day for 3,3′-DMB. The derived $BMDL_{10}$ values are similar in magnitude to the previously published LTD_{10} (the lowest tumorigenic dose associated with a 10% increase in tumour incidence above controls), which is 0.08 mg/kg-bw per day for 3,3′-DMB·2HCl (CPDB 2012).

The majority of *in vitro* and *in vivo* genetic toxicology test results for 3,3′-DMB were positive. *In vivo*, 3,3′-DMB induced micronuclei in rats and mice treated orally by gavage (Cihak 1979; Rajaguru et al. 1999), but not in mice administered 3,3′-DMB by intraperitoneal injection (Morita et al. 1997). Chromosomal aberrations were induced in bone marrow when mice were given 3,3′-DMB by intraperitoneal injection (You et al. 1993), and DNA damage was observed (by comet assay) in all tissues examined from mice following a single oral (gavage) dose (Sasaki et al. 1999). 3,3′-DMB also induced sex-linked recessive mutations in *Drosophila* when given in feed or by injection (NTP 1991b). In mammalian cells *in vitro*, 3,3′-DMB was mutagenic both with and without metabolic activation in mouse lymphoma assays (Mitchell et al. 1988; Myhr and Caspary 1988). DNA and chromosome damage were induced without activation, but mixed results were obtained in the presence of metabolic activation (Martin et al. 1978; Waalkens et al. 1981; Kornbrust and Barfknecht 1984; Barfknecht et al. 1987; Galloway et al. 1987; NTP 1991b). In mutagenicity tests in bacteria (Ames assay), 3,3′-DMB was positive in the frameshift mutation-detecting strains TA98 and 1538 only in the presence of metabolic activation. In other strains (TA97, TA100, TA102, TA1535 and TA1537), negative results were generally observed both with and without activation (Tanaka et al. 1980; Waalkens et al. 1981; Tanaka et al. 1982; Omar 1983; Kennelly et al. 1984b; Prival et al. 1984; Reid et al. 1984; De France et al. 1986; Krishna et al. 1986; Zeiger et al. 1988; NTP 1991b; You et al. 1993; Makena and Chung 2007). Mixed results were obtained in bacterial DNA damage tests (SOS/umu) (Oda et al. 1995; Oda 2004).

The health effects of the 3,3′-DMB-based substances are expected to be similar to those of 3,3′-DMB, due to the release of 3,3′-DMB following azo bond reductive cleavage.

Benzidine-based Dye That May Release 3,3′-DCB

Direct Red 46 releases 3,3′-DCB upon azo bond reductive cleavage (refer to Table 18).

Direct Red 46 was not mutagenic in the Ames assay in the presence or absence of metabolic activation (Joachim and Decad 1984; Reid et al. 1984). Mutagenicity was observed in *Salmonella typhimurium* strains TA98 and TA1538 in the presence of metabolic activation only after reductive conditions, such as Prival modifications, incubations with sodium dithionite or incubations with rat cecal bacteria were incorporated into the assay (Gregory et al. 1981; Reid et al. 1984). One study that used the Prival modifications, however, observed no mutagenicity in TA98 following incubation with the purified dye (ILS 2011b). This may be attributed to concentrations that were not sufficiently high to achieve mutagenicity. Equivocal results were obtained for unscheduled DNA synthesis when the purified dye was either incubated with primary rat hepatocytes or orally administered to rats (Joachim and Decad 1984). DNA damage, however, was clearly detected in the liver of female rats exposed to Direct Red 46 for 4 weeks in their drinking water (Sagelsdorff et al. 1996). No carcinogenicity study was identified for Direct Red 46.

The Government of Canada has evaluated 3,3′-DCB and concluded that it was harmful to human health, based on carcinogenicity and genotoxicity (Canada 1993b). It is also classified as a group 2B carcinogen (possibly carcinogenic to humans). The IARC working group concluded that “there is *sufficient evidence* in experimental animals for the carcinogenicity of 3,3′-dichlorobenzidine” (IARC 1974b, 1982b, 1987b, 2010c). The health effects of Direct Red 46 are expected to be similar to those of 3,3′-DCB, due to the release of 3,3′-DCB following azo bond reductive cleavage.

Benzidine-based Substances That May Release 2,2′-Dimethylbenzidine (2,2′-DMB), 2,2′-Dichlorobenzidine (2,2′-DCB), 2,2′-Disulfobenzidine (2,2′-DSB), 3,3′-Dicarboxybenzidine (3,3′-DCAB) or 3,3′-Di(carboxymethoxy)benzidine (3,3′-DCMB)

Eight of the Benzidine-based Substances release benzidine derivatives that have not been assessed or classified by any national or international agencies (2,2’-DMB; 2,2’-DCB; 2,2’-DSB; 3,3’-DCAB; 3,3’-DCMB). Limited data were identified on these eight Benzidine-based Dyes or the benzidine derivatives that may be released.Therefore, SAR analysis was also considered. The hazard potential for non-benzidine aromatic amines released following azo bond cleavage was considered for one substance for which exposure to the general population is expected (i.e.Acid Red 97) (Table 7-7).

Table 7-7. Benzidine-based Substances that may release 2,2′-DMB, 2,2′-DCB, 2,2′-DSB, 3,3′-DCAB or 3,3′-DCMB

Chemical name/ acronym	Subgroup[a]	Benzidine Derivative released	Non-benzidine aromatic amine(s) released
Acid Red 99	Acid Dyes	2,2′-DMB	CAS RN 2834-92-6 No CAS RN
BADB	Acid Dyes	2,2′-DMB	No CAS RN
BAHSD	Direct Dyes	2,2′-DCB	CAS RN 89-57-6 No CAS RN
Acid Orange 56	Acid Dyes	2,2′-DSB	CAS RN 2834-92-6
Acid Red 97	Acid Dyes	2,2′-DSB	2834-92-6
NAADD	Acid Dyes	2,2′-DSB	CAS RN 615-71-4 CAS RN 62-53-3 No CAS RN
BDAAH	Direct Dyes	3,3′-DCAB	98-32-8 No CAS RN
Direct Blue 158	Direct Dyes	3,3′-DCMB	No CAS RN

Abbreviations: 2,2′-DCB, 2,2′-dichlorobenzidine; 2,2′-DMB, 2,2′-dimethylbenzidine; 2,2′-DSB, 2,2′-disulfobenzidine; 3,3′-DCAB, 3,3′-dicarboxybenzidine; 3,3′-DCMB, 3,3′-di(carboxymethoxy)benzidine; n.d., no data; SAR, structure–activity relationship

[a] Acid Dyes and Direct Dyes refer to the Benzidine-based Acid Dyes and Benzidine-based Direct Dyes subgroups, respectively.

Benzidine-based Substances That May Release 2,2′-DMB or 2,2′-DCB

Acid Red 99 and BADB release 2,2′-dimethylbenzidine (2,2’-DMB; CAS RN 84-67-3) following azo bond reductive cleavage. In addition, data on Acid Red 111 (which also releases 2,2’-DMB) were included to inform the health effects assessment. BAHSD releases 2,2′-dichlorobenzidine (2,2’-DCB; CAS RN 84-68-4) following azo bond reductive cleavage.

In vitro genotoxicity assays indicate that Acid Red 111 and Acid Red 99 are mutagenic only in the presence of reductive conditions and metabolic activation in the *Salmonella* mutagenicity assay (Venturini and Tamaro 1979; Gregory et al. 1981; Zhou et al. 1987; ILS 2011a). Acid Red 111 was negative for bacterial DNA damage, but no reductive conditions were used in the assay (Kosaka and Nakamura 1990). BADB, however, was not mutagenic following reductive metabolism and metabolic activation in both TA98 and TA100 in the *Salmonella* mutagenicity assay for doses up to 1750 µg/plate (ILS 2011b). The top dose was considerably less than the 5 mg/plate recommended by OECD guidelines (OECD 1997), and it is therefore possible that the dye may be mutagenic at higher doses. No data were identified for BAHSD.

2,2′-DMB was mutagenic in TA98 and TA100 with metabolic activation, and 2,2′-DCB was positive for unscheduled DNA synthesis in HeLa cells (Martin et al. 1978; Hinks et al. 2000). Similar results were obtained in genotoxicity assays conducted for 3,3′-DMB and 3,3′-DCB, and there is no evidence to suggest that changing the position of the methyl group or the chlorine atom from the *ortho* to the *meta* position would eliminate mutagenicity.

The health effects of these four Benzidine-based Dyes are expected to be similar to those of 2,2-DMB and 2,2’-DCB, due to the release of 2,2-DMB or 2,2’-DCB following azo bond reductive cleavage.

Benzidine-based Substances That May Release 2,2′-DSB

Acid Red 97, Acid Orange 56 and NAADD release 2,2′-disulfobenzidine (2,2′-DSB; CAS RN117-61-3) following reductive cleavage of the azo bond.

Mixed results were obtained in *in vitro* mutagenicity assays for Acid Red 97: in one study, Acid Red 97 was mutagenic in the presence of reductive conditions and metabolic activation in TA98 and TA100, while in another, it was not (Gregory et al. 1981; ILS 2011a). An *in vitro* mutagenicity study conducted under reductive conditions was also identified for Acid Orange 56, but it was disregarded due to the low purity of the dye (41.8%) (ILS 2011a).

In vitro genotoxicity studies indicate that 2,2′-DSB is not mutagenic in five strains of *Salmonella typhimurium* with and without metabolic activation as well as with and without reductive conditions (ETAD 1989; NTP 1993; ILS 2011a). Furthermore, available data pertaining to other sulfonated aromatic amines indicate that these substances generally have no or very low genotoxic effects. In a review, Jung et al. (1992) showed that mutagenicity seen in several aromatic amines is absent in their corresponding sulfonated analogues. This was discussed for benzidines specifically in a review by Chung et al. (2006). Several groups have also postulated mechanistic considerations that may be responsible for the mitigating effect of sulfonation on the mutagenic potential of aromatic amines, including increased electronegativity and water solubility (Marchisio et al. 1976; Lin and Solodar 1988; OECD QSAR Toolbox 2011).

The other postulated azo bond cleavage product of Acid Red 97 is 1-amino-2-napthol (CAS RN 2834-92-6). The available data on this aromatic amine indicates that it is not directly mutagenic *in vitro*. (Garner and Nutman 1977; Chung et al. 1981; Freeman et al. 1987; Dillon et al. 1994)

Data on other endpoints were not available for Acid Red 97 or its metabolite 2,2'-DSB. Limited data were available on the metabolite 1-amino-2-napthol; however, the azo acid dye Orange II also releases this aromatic amine upon azo cleavage. In oral repeated-dose studies on Orange II in rats, spleen and blood effects were consistently observed, characteristic of amine-induced anemia (Hamann et al. 2000; Rofe 1957; Rosner 1999a,b; Singh and Khanna 1979; Singh et al. 1987). Effect levels in these studies ranged from 10 to 250 mg/kg-bw per day. No effects were observed in a skin painting study in which mice received weekly uncovered skin applications of Orange II for 18 months at approximately 5 mg/kg-bw per day (Carson 1984).

Benzidine-based Substance That May Release 3,3′-DCAB

BDAAH may release 3,3′-dicarboxybenzidine (3,3′-DCAB; CAS RN 2130-56-5) following azo bond reductive cleavage. No empirical data were identified for this dye. No empirical data were identified for the benzidine derivative 3,3′-DCAB. According to the Benigni/Bossa rulebase for mutagenicity and carcinogenicity, aromatic amines with a carboxylic acid group *ortho* to the nitrogen substituent may have diminished mutagenic potential (Benigni et al. 2008). The authors postulated that the carboxylic acid group in this position may hinder the metabolic activation of the nitrogen substituent to its reactive metabolite. This is further supported by studies on other aromatic amines (Takagi et al. 1995; ECJRC 2001).

Benzidine-based Substance That May Release 3,3′-DCMB

Direct Blue 158 releases 3,3′-di(carboxymethoxy)benzidine (3,3′-DCMB; CAS RN 3366-63-0) following azo bond reductive cleavage.

Direct Blue 158 was not mutagenic in *Salmonella typhimurium* strain TA98 or TA100 in the presence of metabolic activation alone or when reductive conditions and metabolic activation were incorporated into the bacterial mutagenicity assay (ILS 2011a).

No empirical data were identified for 3,3′-DCMB. No existing SAR analysis looking specifically at aromatic amines substituted with a glycolic acid group was identified. The presence of electron-donating groups in the *ortho* position to the amine (e.g., the divalent oxygen of the glycolic acid group) may promote the activation of the aromatic amine by stabilizing the nitrenium ion through delocalization of charge in the aromatic ring system. However, the glycolic acid group may also hinder *N*-hydroxylation (a precursor metabolic step in the formation of the nitrenium ion) due to steric hindrance at the adjacent *ortho* position. The glycolic acid group will also increase the water solubility of benzidine, which would favour the elimination of unchanged chemical and make *N*-hydroxylation (a precursor metabolic step in the formation of the nitrenium ion) less likely to occur (OECD 2011; DEREK Nexus 2010).

Benzidine-based Substances Without Azo Bond

The Benzidine-based Cationic Indicators TDBPD and TBDB and the Benzidine-based Precursor Naphthol AS-BR do not contain azo bonds. Therefore, the azo bond reduction mechanism of toxicity in which the benzidine derivative may be released as a metabolite through azo bond cleavage is not applicable to these substances.

TDBPD was negative for mutagenicity in all strains of *Salmonella* and *Escherichia coli* tested with or without metabolic activation (Venitt and Crofton-Sleigh 1979). It was also negative in the *in vitro* chromosomal aberration assay in Chinese hamster ovary cells with and without metabolic activation (Au and Hsu 1979). Simulated mammalian liver metabolism on the neutral form (non-chloride) of TDBPD was conducted using the software OASIS TIMES Mix version 2.27.3 (Mekenyan et al. 2004). The preferred transformation of TDBPD involved the dealkylation of the methoxy group(s) to a hydroxyl group followed by sulfation or *O*-glucuronidation. No release of 3,3′-DMOB was predicted. However, since the structure was not in the domain of the model, the confidence in this prediction is considered low.

TDBD was positive in the Ames assay under reductive conditions in the presence of metabolic activation, but showed equivocal results in the presence of metabolic activation alone in *Salmonella typhimurium* strain TA98. The maximum response in TA98 with and without FMN was seen at the lowest dose tested, and the response decreased with higher doses. TDBD was also negative in TA100 with and without reductive conditions (ILS 2011a). Simulated mammalian liver metabolism on the neutral form (non-chloride) of TDBD was conducted using the software OASIS TIMES Mix version 2.27.3 (Mekenyan et al. 2004). The preferred transformation of TDBD involved the dealkylation of the methoxy group(s) to a hydroxyl group followed by sulfation or *O*-glucuronidation. No release of 3,3′-DMOB was predicted. Since the structure was not in the domain of the model, the confidence in this prediction is considered low.

No empirical data were identified for Naphthol AS-BR Simulated S9-mediated metabolism of Naphthol AS-BR was conducted using the software OASIS TIMES Mix version 2.27.3 (Mekenyan et al. 2004). The metabolic tree created by the TIMES simulator indicates that the preferred metabolic transformation is *O*-glucuronidation or sulfation at the hydroxyl position on the naphthalene ring. No amide bond hydrolysis takes place, and therefore 3,3′-DMB is not released in the simulation. However, the structure was out of the model's domain, indicating that the reliability of this prediction should be considered low. The physical and chemical properties of Naphthol AS-BR were also analysed; this substance was predicted not to be bioavailable due to its high log K_{ow} (> 5) and its high molecular weight (> 500 Da), according to the Lipinski Rule of Five (Lipinski et al. 2001). Considering the likely low potential bioavailability of this compound, amide bond hydrolysis *in vivo* does not seem likely. Therefore, 3,3′-DMB is not likely released due to the metabolism of this compound.

Benzidine Derivatives: TODI and 4N-TMB

TODI

In a mutagenicity study in bacteria, TODI was found to be mutagenic in *Salmonella typhimurium* strains TA98 and TA1538 in the presence, but not in the absence, of metabolic activation. This Benzidine Derivative was negative in *Salmonella typhimurium* strains TA100, TA1535 and TA1537 and in *Escherichia coli* WP2*uvrA*, with or without metabolic activation. In the same study, TODI induced chromosomal aberrations in Chinese hamster lung cells in the presence, but not in the absence, of metabolic activation (JETOC 1996). In unpublished reports identified as submissions to REACH in the EU, TODI was positive for gene mutation in mammalian cells in the presence, but not in the absence, of metabolic activation; negative for unscheduled DNA synthesis in hepatocytes of rats given a single dose by oral gavage; and negative for micronuclei in the bone marrow of mice treated once by intraperitoneal injection (ECHA 2012). All of the studies identified for this Benzidine Derivative were from secondary sources; therefore, the reliability is uncertain.

The *in vitro* genotoxicity test results for TODI are consistent with the results for other benzidine derivatives, including 3,3′-DMOB and 3,3′-DMB. In addition, the related benzidine derivative 3,3′-dimethoxybenzidine-4,4′-diisocyanate (CAS RN 91-93-0), which can be hydrolysed to 3,3′-DMOB, was mutagenic in Ames tests in strain TA98 with metabolic activation and was carcinogenic to rats in a 2-year oral bioassay (NTP 1979; IARC 1986). As the only difference between 3,3′-dimethoxybenzidine-4,4′-diisocyanate and TODI is the change from methoxy groups to methyl groups at the 3,3′-positions, this implies that TODI could similarly be hydrolyzed to 3,3′-DMB.

4N-TMB

In vitro, 4N-TMB was mutagenic in *Salmonella typhimurium* strain TA98 with metabolic activation in two studies. In the same studies, negative results were obtained in strain TA100, both with and without metabolic activation, and in strain TA98 without activation (Messerly et al. 1987; Chung et al. 2000). In a third bacterial mutagenicity study, negative results were obtained with *Salmonella* strains TA98, TA100, TA1535 and

TA1537, both with and without activation (McCann et al. 1975). McConlogue et al. (1980) reported in an abstract that 4N-TMB was negative in a sister chromatid assay in Chinese hamster ovary cells. However, the authors also reported that the test compound was insoluble at concentrations equivalent to those at which benzidine induced sister chromatid exchanges.One research group has reported the peroxidase-catalyzed oxidative *N*-demethylation of 4N-TMB (O'Brien and Gregory 1985; McGirr and O'Brien 1987) and observed that the products of this biotransformation can bind DNA.

7.3 Characterization of Risk to Human Health

Exposure of the general population of Canada to Benzidine-based Substances and Benzidine Derivatives from environmental media is not expected due to limited commercial quantities in Canada; therefore risk from these sources is not expected.

7.3.1 Benzidine Derivatives

A review of the available health effects data on 3,3′-DMB and 3,3′-DMOB indicates strong evidence for the mutagenicity and carcinogenicity of these substances. In rat studies, tumours were observed at less than lifetime exposure durations and in both sexes at multiple sites. The mode of action of carcinogenicity is relatively well understood and likely occurs through the direct interaction of reactive intermediates with DNA. 3,3′-DMB·2HCl is expected to dissociate in physiological media and is therefore considered to be toxicologically equivalent to 3,3′-DMB. As such, the health effects information regarding 3,3′-DMB is considered applicable for 3,3′-DMB·2HCl.

In Europe and Japan, 3,3′-DMB and 3,3′-DMOB were detected in some textiles and leather products, some of which were reported to be imported from other countries, and may therefore be present in imported products in Canada, as the Canadian textile market is predominantly composed of imported products. Testing of products on the Canadian market, however, did not identify these two Benzidine Derivatives in imported and domestic textile products (Health Canada 2013). Overall, exposure to 3,3′-DMB and 3,3′-DMOB from textiles and leather is considered to be limited; direct and prolonged skin contact is not expected. Therefore, risk to human health for the general population from use of textile or leather products is not expected.

Potential daily oral exposure to 3,3′-DMB from use of polyamide cooking utensils was conservatively estimated to range from 2×10^{-6} mg/kg-bw per day (12 years of age and older) to 6.5×10^{-6} mg/kg-bw per day (toddlers 0.5–4 years of age). The critical effect level for 3,3′-DMB is a BMDL_{10} of 0.38 mg/kg-bw per day, based on skin basal cell neoplasms in male F344/N rats in a chronic oral study. Comparison of the upper-bounding estimate of oral exposure to 3,3′-DMB with the critical effect level results in an MOE greater than 58,000, which is considered adequate to address uncertainties in the health effects and exposure databases.

Since 3,3′-DMB·2HCl, TODI and 4N-TMB are used as intermediates in chemical synthesis, the presence of these substances in Canadian or imported products is not expected, except potentially as residuals at very low levels. Exposure of the general population to these substances is not expected; therefore the risk to human health is not expected.

7.3.2 Benzidine-based Substances

The genotoxic and carcinogenic properties of benzidine-based dyes are due to the release of free benzidine, benzidine derivatives and other aromatic amines via reductive cleavage of the azo bond and subsequent conversion of these substances to reactive electrophilic intermediates (Environment Canada and Health Canada 2013). The human health effects of these substances are therefore assessed based on their capacity to undergo reductive azo bond cleavage and on the genotoxic and carcinogenic properties of the aromatic amines released. All of the available *in vivo* and *in vitro* metabolism data as well as reductive Ames test results for the Benzidine-based Substances with azo bonds support the potential for reductive cleavage. Two Benzidine-based Cationic Indicators and one Benzidine-based Precursor do not have azo bonds.

Benzidine-based Substances That May Release Benzidine, 3,3′-DCB, 3,3′-DMOB or 3,3′-DMB

Following reductive azo bond cleavage, 26 Benzidine-based Substances are considered to have the potential to release benzidine or one of three other EU22 benzidine derivatives (3,3′-DCB, 3,3′-DMOB and 3,3′-DMB) (Table 7-3). Benzidine and 3,3′-DCB have been previously assessed by the Government of Canada, while 3,3′-DMOB and 3,3′-DMB are substances currently being evaluated in this assessment. A review of the available data on these four benzidine derivatives clearly shows that they are carcinogenic and mutagenic. In addition, empirical data for genotoxicity are available for approximately half of the Benzidine-based Substances being evaluated in this assessment, and carcinogenicity data are available for five of these substances. Where data are available, the genotoxicity and carcinogenicity databases of the Benzidine-based Substances are consistent with those of the corresponding benzidine derivative. The potential health effects of these 26 Benzidine-based Substances can be attributed to the toxicity of the benzidine derivative released.

Based on the available information, no manufacture or import activities above the reporting threshold in Canada was confirmed for any of these Benzidine-based Substances. Direct Blue 14 was reported to be imported in Canada in the DSL Inventory Update survey for use as a laboratory substance in quantities below or equal to the reporting threshold. The four EU22 aromatic amines were occasionally detected in imported textiles and leather products in Europe and Japan and may therefore be present in imported products in Canada, as the Canadian textile market is predominantly composed of imported products. Testing of products on the Canadian market, however, did not identify these four aromatic amines in imported and domestic textile and leather products (Health Canada 2013). The detection of an EU22 aromatic amine does not indicate the presence or absence of an individual dye, as specific dyes are not identified by the testing method.

Exposure of the general population to any of these Benzidine-based Substances from textiles and leather is considered to be limited; direct and prolonged skin contact is not expected. Therefore, risk to human health is not expected.

Benzidine-based Substances that May Release 2,2′-DMB, 2,2′-DCB, 2,2′-DSB, 3,3′-DCAB or 3,3′-DCMB

There are three Benzidine-based Substances that may release 2,2′-DMB or 2,2′-DCB, following reductive cleavage of the azo bond (Table 7-4). It is considered that the benzidine derivatives 2,2′-DMB and 2,2′-DCB may be oxidized to active intermediates through the same pathway as for benzidine, 3,3′-DMB and 3,3′-DCB. The potential health effects of these three Benzidine-based Substances can be attributed to the toxicity of the benzidine derivative released. There are five Benzidine-based Substances that may release 2,2′-DSB, 3,3′-DCAB or 3,3′-DCMB, following reductive cleavage of the azo bond (Table 7-4). Limited international data is available on the presence of non-EU22 aromatic amines in textiles. Testing of products on the Canadian market did not identify the aromatic amines 2,2′-DMB or 2,2′-DCB in imported and domestic textile and leather products (Health Canada 2013).

Only one substance (Acid Red 97) had confirmed commercial activity in Canada based on information from industry (2010 email from ETAD to Program Development and Engagement Division, Environment Canada; unreferenced). The major use of Acid Red 97 is to dye leather and textile materials, which may potentially be used for consumer products that lead to direct exposure. Conservative estimates of dermal exposure to Acid Red 97 due to skin contact with textile clothing and leather articles range from 0.0026 to 0.0040 mg/kg-bw per day and from 2.1×10^{-3} to 7.7×10^{-2} mg/kg-bw, respectively (see Appendix E). Based on the available data, carcinogenicity and genotoxicity are not expected to be endpoints of concern for Acid Red 97, and the hazard potential is low. Therefore, risk to human health for the general population from Acid Red 97 in textiles and leather is considered to be low.

Exposure of the general population to any of the remaining Benzidine-based Substances from textiles and leather is considered to be limited; direct and prolonged skin contact is not expected. Therefore, risk to human health is not expected.

Benzidine-based Substances Without Azo Bond

The three Benzidine-based Substances without an azo bond are not expected to result in direct exposure of the general population due to their general use as laboratory reagents and chemical precursors. In addition, none of these three Benzidine-based Substances are expected to release aromatic amines by azo bond reductive cleavage. Therefore, risk to human health is not expected.

7.3.3 Uncertainties in Evaluation of Risk to Human Health

Acid Red 97 was identified in a list of dyes known by ETAD as being sold or used in Canada (personal communication, email from ETAD to Environment Canada, dated 2010; unreferenced); however, the specific use of Acid Red 97 in Canada was not indicated. It is assumed, based on the information available elsewhere (CII 2013), that Acid Red 97 is used as a dye for textiles and leather that may come in contact with consumers in Canada.

The detection of the Benzidine Derivatives 3,3′-DMB and 3,3′-DMOB in the product surveys (EurAzos 2007; Kawakami et al. 2010; RAPEX 2012) is specific primarily to imported textile and leather products in the European and Japanese markets, and uncertainty exists in the potential exposure of the general population of Canada to these textile and leather products based on these data. Uncertainty is also recognized in the results of testing of products on the Canadian market (Health Canada 2013), which involved a limited number of samples. Despite the uncertainties, the results are consistent with the global phase out of dyes based on EU22 aromatic amines, which include 3,3′-DMB and 3,3′-DMOB.

Exposure estimates presented in this assessment assume conditions of intended or reasonably foreseeable use behaviours resulting in direct and/or prolonged exposure. They are developed for use of polyamide cooking utensils, dermal contact with textile apparel, mouthing of textile objects (e.g., toys and blankets) by infants and dermal contact with leather articles (e.g., apparel, furniture, and children's toys). These exposure scenarios are considered to represent the predominant sources of exposure based on use patterns. There exists uncertainty in some of the parameter inputs used in estimating exposures. However, conservative assumptions were used to yield upper-bounding exposure estimates, such as considering full adult body coverage for dermal exposure via textile apparel.

Since Acid Red 97 is relatively water soluble, the effect of laundering is expected to significantly reduce any dye that is not fixed to the textile fibre, thereby reducing the exposure after the initial washes. This effect was not factored in the derivation of exposure; therefore the estimated exposures to Acid Red 97 from textiles are conservative.

Overall confidence in the health effects database ranges from low to high for the Benzidine-based Substances and Benzidine Derivatives in this assessment. For the Benzidine-based Substances that were evaluated for azo bond cleavage, confidence is high that aromatic amines are released. Confidence is also high in the approach taken in which the potential hazard of aromatic amine metabolites was used to infer the potential hazard of the Benzidine-based Substances. However, uncertainty is recognized in association with the identity of the metabolites and the relative efficiency of *in vivo* azo reductive cleavage.

For approximately one-third of these substances, all of which are based on benzidine, 3,3′-DMOB, 3,3′-DMB or 3,3′-DCB, the identity of the metabolites produced in vivo was confirmed. The relative efficiency of azo bond reductive cleavage *in vivo* is considered highly variable, and uncertainty around the degree of cleavage *in vivo* also results in uncertainty in the amount of aromatic amine metabolites released.

There is more uncertainty in the health effects databases for the Benzidine-based Substances based on benzidine derivatives other than benzidine, 3,3′-DMOB, 3,3′-DMB or 3,3′-DCB. Those substances were generally more data poor; for example, azo bond cleavage was inferred from reductive Ames tests or by general read-across: no *in vivo* or *in vitro* metabolism studies were available to confirm the presence or identity of aromatic amine metabolites, including the benzidine derivatives. Empirical hazard data were limited to *in vitro* mutagenicity assays for the substances or metabolites. Further mutagenicity testing of parent Benzidine-based Substances under reductive conditions would increase the confidence in the health effects database for data-poor Benzidine-based Substances.

The carcinogenic potential of benzidine has been demonstrated in humans. As the pathway for tumour induction is suspected to be the same for Benzidine Derivatives, including 3,3′-DMOB and 3,3′-DMB, it is reasonable to assume that 3,3′-DMOB and 3,3′-DMB are also potential human carcinogens. Similarly, it is reasonable to assume that substances capable of releasing benzidine and the Benzidine Derivatives 3,3′-DCB, 3,3′-DMB and 3,3′-DMOB upon reductive cleavage of the azo bond are potential human carcinogens. For the Benzidine-based Substances that release 2,2′-DMB and 2,2′-DCB, it is possible that tumours could be induced in humans by the same pathway as those based on benzidine. However, there is not enough information in the toxicity database to determine the carcinogenic potential of these dyes with confidence.

There is also uncertainty associated with substance purity; in many instances, the purity of the substance used in experimental studies was not reported, or the substance was reported to contain a number of impurities, including their corresponding benzidine and other aromatic amine precursors.

7.3.4 Benzidine Derivatives and Benzidine-based Substances with Human Health 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 Benzidine Derivatives, Benzidine-based Acid Dyes, Benzidine-based Direct Dyes, and Benzidine-based Precursors in this assessment have human health effects of concern based on potential carcinogenicity. These include the Benzidine Derivatives 3,3′-DMOB, 3,3′-DMB and 3,3′-DMB·2HCl, as well as those Benzidine-based Substances which may release benzidine, 3,3′-dichlorobenzidine (3,3′-DCB), 3,3′-DMB, 3,3′-DMOB, 2,2′-dimethylbenzidine (2,2′-DMB) or 2,2′-dichlorobenzidine (2,2′-DCB). A list of these substances with human health effects of concern based on potential carcinogenicity are shown in Appendix H.

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 42 Benzidine-based Dyes and Related Substances evaluated in this assessment. It is concluded that these Benzidine-based Dyes and Related Substances 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 information presented in this Screening Assessment, it is concluded that the Benzidine-based Dyes and Related Substances 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.

It is concluded that the Benzidine-based Dyes and Related Substances evaluated in this assessment do not meet any of the criteria set out in section 64 of CEPA 1999.

References

ACD/I-Lab [Prediction Module]. 2010–2011. Toronto (ON): Advanced Chemistry Development. [cited 2012 Nov]. Available from: www.acdlabs.com/resources/ilab/ [restricted access]

[ACGIH] American Conference of Governmental Industrial Hygienists. 1986. Documentation of the threshold limit values. 5th ed. Cincinnati (OH): ACGIH. p. 577.

Acros Organics. 2006. Material Safety Data Sheet: Tartrazine (1934-21-0) [Internet]. Fair Lawn (NJ): MSDSonline. [cited 2011 September]. Available from: www.msdsonline.com [restricted access]

Acros Organics. 2007. Material Safety Data Sheet: 3,3'-Dimethoxybenzidine (119-90-4) [Internet]. Fair Lawn (NJ): MSDSonline. [cited 2011 September]. Available from: www.msdsonline.com [restricted access]

Acros Organics. 2008. Material Safety Data Sheet: N,n,n',n'-tetramethylbenzidine (366-29-0) [Internet]. Fair Lawn (NJ): MSDSonline. [cited 2011 September]. Available from: www.msdsonline.com [restricted access]

Acros Organics. 2009a. Material Safety Data Sheet: Metanil yellow (tech.), 85% (uv-vis) [Internet]. Fair Lawn (NJ): MSDSonline. [cited 2011 September]. Available from: www.msdsonline.com [restricted access]

Acros Organics. 2009b. Material Safety Data Sheet: Orange (II) (Cert) [Internet]. Fair Lawn (NJ): MSDSonline. [cited 2011 September]. Available from: www.msdsonline.com [restricted access]

[Alfa Aesar] Interactive Alfa Aesar Database [database on the Internet]. ©2011. Ward Hill (MA): Alfa Aesar. [cited 2011]. Available from: www.alfa.com/en/go160w.pgm?srchtyp=product

Amacher DE, Zelljadt I. 1983. The morphological transformation of Syrian hamster embryo cells by chemicals reportedly nonmutagenic to *Salmonella typhimurium*. Carcinogenesis 4(3):291–295.

Anliker R, Clarke EA, Moser P. 1981. Use of the partition coefficient as an indicator of bioaccumulation tendency of dyestuffs in fish. Chemosphere 10(3):263–274.

[AOPWIN] Atmospheric Oxidation Program for Windows [Estimation Model]. 2010. Version 1.92a. Washington (DC): US Environmental Protection Agency, Office of

Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

Applegate VC, Howell JH, Hall AE Jr, Smith MA. 1957. Toxicity of 4,346 chemicals to larval lampreys and fishes. (Special Scientific Report: Fisheries No. 207). Washington (DC): US Fish and Wildlife Service. p. 157.

Arnot JA, Gobas FAPC. 2003. A generic QSAR for assessing the bioaccumulation potential of organic chemicals in aquatic food webs [Internet]. QSAR Comb Sci 22(3):337–345. Available from: www3.interscience.wiley.com/journal/104557877/home [restricted access]

[ATSDR] Agency for Toxic Substances and Disease Registry. 2001. Toxicological profile for benzidine. Atlanta (GA): US Department of Health and Human Services, Public Health Service. Available from: www.atsdr.cdc.gov/toxprofiles/tp62.pdf

Au W, Hsu TC. 1979. Studies on the clastogenic effects of biologic stains and dyes. Environ Mutagen 1(1):27–35.

Aznar M, Vera P, Canellas E, Nerín C, Mercea P, Störmer A. 2011. Composition of the adhesives used in food packaging multilayer materials and migration studies from packaging to food. J Mater Chem 21:4358–4370.

Bafana A, Chakrabarti T, Muthal P, Kanade G. 2009a. Detoxification of benzidine-based azo dye by *E. gallinarum*: Time-course study. Ecotoxicol Environ Saf 72:960–964.

Bafana A, Jain M, Agrawal G, Chakrabarti T. 2009b. Bacterial reduction in genotoxicity of Direct Red 28 dye. Chemosphere 74(10):1404–1406.

Baird R, Carmona L, Jenkins RL. 1977. Behaviour of benzidine and other aromatic amines in aerobic wastewater treatment. Water Pollut Control Fed 49(7):1609–1615.

Barfknecht TR, Naismith RW, Kornbrust DJ. 1987. Variations on the standard protocol design of the hepatocyte DNA repair assay. Cell Biol Toxicol 3(2):193–207.

Baughman GL, Perenich TA. 1988. Investigating the fate of dyes in the environment. Am Dyest Rep 7(2):19–20, 22, 47–48. [cited in Øllgaard et al. 1998].

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

[BCFBAF] Bioaccumulation Program for Windows [Estimation Model]. 2010. Version 3.10. Washington (DC): US Environmental Protection Agency, Office of Pollution

Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

Beije B. 1987. Induction of unscheduled DNA synthesis in liver and micronucleus in bone marrow of rats exposed *in vivo* to the benzidine-derived azo dye, Direct Black 38. Mutat Res 187(4):227–234.

Beilstein FK. 1984. Beilstein handbook of organic chemistry. Berlin (DE): Springer-Verlag.

Benigni R, Bossa C, Jeliazkova N, Netzeva T, Worth A. 2008. The Benigni/Bossa rulebase for mutagenicity and carcinogenicity—a module of Toxtree. European Commission, Joint Research Centre, Institute for Health and Consumer Protection. Report No.: EUR 23241 EN.

[BfR] Federal Institute for Risk Assessment. 2007. Introduction to the problems surrounding garment textiles [Internet]. (BfR Information No. 018/2007). Available from: www.bfr.bund.de/cm/349/introduction_to_the_problems_surrounding_garment_textiles.pdf

[BIOWIN] Biodegradation Probability Program for Windows [Estimation Model]. 2008. Version 4.10. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

Birner G, Albrecht W, Neumann H. 1990. Biomonitoring of aromatic amines. III: Hemoglobin binding of benzidine and some benzidine congeners. Arch Toxicol 64(2):97–102.

Bos RP, Groenen MAM, Theuws JLG. 1984. Metabolism of benzidine-based dyes and the appearance of mutagenic metabolites in urine of rats after oral or intraperitoneal administration. Toxicology 31(3–4):271–282.

Bos RP, Van der Krieken W, Smeijsters L. 1986a. Internal exposure of rats to benzidine derived from orally administered benzidine-based dyes after intestinal azo reduction. Toxicology 40(2):207–213.

Bos RP, Koopman JP, Theuws JLG, Kennis HM, Henderson PT. 1986b. Appearance and reappearance of mutagens in urine from rats after oral administration of Direct Brown 95, due to corprophagy. Toxicology 39(1):85–92.

Bowman MC, King JR, Holder CL. 1976. Benzidine and congeners: analytical chemical properties and trace analyses in five substrates. Int J Environ Anal Chem 4(3):205–223 [cited in Canada 1993a].

Bowman MC, Oller WL, Nony CR. 1982. Metabolism and distribution of two ^{14}C-benzidine-congener-based dyes in rats as determined by GC, HPLC, and radioassays. J Anal Toxicol 6(4):164–174.

Bowman MC, Nony CR, Billedeau SM. 1983. Metabolism of nine benzidine-congener-based azo dyes in rats based on gas chromatographic assays of the urine for potentially carcinogenic metabolites. J Anal Toxicol 7(1):55–60.

Brown D. 1992. Environmental assessment of dyestuffs—Disperse dyes data summaries. Basel (CH): Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers.

Brown D, Hamburger B. 1987. The degradation of dyestuffs: Part III—Investigations of their ultimate degradability. Chemosphere 16(7):1539–1553.

Brown D, Laboureur P. 1983. The degradation of dyestuffs: Part I—Primary biodegradation under anaerobic conditions. Chemosphere 12(3):397–404.

Brown DV. 1963a. Subcutaneous neoplasms induced by trypan blue. Lab Invest 12:1221–1227.

Brown DV. 1963b. Subcutaneous neoplasms induced by trypan blue. Acta Unio Int Contra Cancrum 19:655–666.

Brown DV, Thorson TA. 1956. Reticulum-cell sarcoma of rats. Experimental production with trypan blue. J Natl Cancer Inst 16(5):1181–1195.Brown JP, Dietrich PS. 1983. Mutagenicity of selected sulfonated azodyes in the *Salmonella*/microsome assay: use of aerobic and anaerobic activation procedures. Mutat Res 116(3–4):305–315.

Brown JP, Roehm GW, Brown RJ. 1978. Mutagenicity testing of certified food colors and related azo, xanthene and triphenylmethane dyes with the *Salmonella*/microsome system. Mutat Res 56(3):249–272.

Cai Y, Pailthorpe MT, David SK. 1999. A new method for improving the dyeability of cotton with reactive dyes. Textile Res J 69:440–446.

Cameron TP, Hughes TJ, Kirby PE, Fung VA, Dunkel VC. 1987. Mutagenic activity of 27 dyes and related chemicals in the *Salmonella*/microsome and mouse lymphoma TK+/− assays. Mutat Res 189(3):223–261.

Canada. 1993a. Benzidine [Internet]. (Priority substances list assessment report). Ottawa (ON): Environment Canada; Health Canada. Available from: www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl1-lsp1/benzidine/index-eng.php

Canada. 1993b. 3,3′-Dichlorobenzidine [Internet]. (Priority substances list assessment report). Ottawa (ON): Environment Canada; Health Canada. Available from: www.hc-sc.gc.ca/ewh-semt/alt_formats/hecs-sesc/pdf/pubs/contaminants/psl1-lsp1/3-dichlorobenzidine/3-dichlorobenzidine-eng.pdf

Canada. 1999. *Canadian Environmental Protection Act, 1999*. S.C., 1999, c. 33. Part III. vol. 22, no. 3. Available from: www.gazette.gc.ca/archives/p3/1999/g3-02203.pdf

Canada. 2000. *Canadian Environmental Protection Act, 1999: Persistence and Bioaccumulation Regulations,* P.C. 2000-348, 23 March 2000, SOR/2000-107. Available from: www.gazette.gc.ca/archives/p2/2000/2000-03-29/pdf/g2-13407.pdf

Canada, Dept. of the Environment, Dept. of Health. 2006a. *Canadian Environmental Protection Act, 1999*: *Notice of intent to develop and implement measures to assess and manage the risks posed by certain substances to the health of Canadians and their environment*. Canada Gazette, Part I, vol. 140, no. 49, p. 4109–4117. Available from: http://gazette.gc.ca/archives/p1/2006/2006-12-09/pdf/g1-14049.pdf

Canada, Dept. of the Environment, Dept. of Health. 2006b. *Canadian Environmental Protection Act, 1999*: *Notice with respect to selected substances identified as priority for action*. Canada Gazette, Part I, vol. 140, no. 9, p. 435–459. Available from: www.gazette.gc.ca/archives/p1/2006/2006-03-04/pdf/g1-14009.pdf

Canada, Dept. of the Environment, Dept. of Health. 2008a. *Canadian Environmental Protection Act, 1999: Notice of sixth release of technical information relevant to substances identified in the Challenge.* Canada Gazette, Part I, vol. 142, no. 22, p. 1639–1644. Available from: http://gazette.gc.ca/rp-pr/p1/2008/2008-05-31/pdf/g1-14222.pdf

Canada, Dept. of the Environment. 2008b. *Canadian Environmental Protection Act, 1999: Notice with respect to Batch 6 Challenge substances.* Canada Gazette, Part I, vol. 142, no. 22, p. 1644–1662. Available from: http://gazette.gc.ca/rp-pr/p1/2008/2008-05-31/pdf/g1-14222.pdf

Canada, Dept. of the Environment. 2009. *Canadian Environmental Protection Act, 1999: Notice with respect to certain inanimate substances (chemicals) on the Domestic Substances List*. Canada Gazette, vol. 143, no. 40. Available from: www.gazette.gc.ca/rp-pr/p1/2009/2009-10-03/html/notice-avis-eng.html#d101

Canada, Dept. of the Environment. 2011. *Canadian Environmental Protection Act, 1999: Notice with respect to certain aromatic amines and aromatic azo- and benzidine-based substances.* Canada Gazette, Part I, vol. 145, no. 51. Available from: http://gazette.gc.ca/rp-pr/p1/2011/2011-12-17/html/sup-eng.html

Canada. 2012. *Canadian Environmental Protection Act, 1999: Prohibition of Certain Toxic Substances Regulations, 2012.* Canada Gazette, Part I, vol. 145, no. 30. Available from: www.gazette.gc.ca/rp-pr/p1/2011/2011-07-23/html/reg1-eng.html

Carson S. 1984. Skin painting studies in mice on 11 FD&C and D&C colors: FD&C Green No. 3, Red No. 2, Red No. 4, Yellow No. 6, and external D&C No. 7, D&C Orange No. 4, Violet No. 2, Red No. 17, Red No. 34, and Yellow No. 7. J Toxicol Cutan Ocul Toxicol 3(3):309–331.

CATALOGIC [Computer Model]. ©2004–2011. Version 5.11.6. Bourgas (BG): Bourgas Prof. Assen Zlatarov University, Laboratory of Mathematical Chemistry. Available from: http://oasis-lmc.org/?section=software&swid=1

[CCRIS] Chemical Carcinogenesis Research Information System [database on the Internet]. 2012. Bethesda (MD): National Library of Medicine (US). [cited 2012 Jul 16]. Available from: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS

Cerniglia CE, Freeman JP, Franklin W, Pack LD. 1982a. Metabolism of benzidine and benzidine-congener based dyes by human, monkey and rat intestinal bacteria. Biochem Biophys Res Commun 107(4):1224–1229.

Cerniglia CE, Freeman JP, Franklin W, Pack LD. 1982b. Metabolism of azo dyes derived from benzidine, 3,3′-dimethylbenzidine and 3,3′-dimethoxybenzidine to potentially carcinogenic aromatic amines by intestinal bacteria. Carcinogenesis 3(11):1255–1260.

Cerniglia CE, Zhou Z, Manning BW. 1986. Mutagenic activation of the benzidine-based dye Direct Black 38 by human intestinal microflora. Mutat Res 175(1):11–16.

ChemBioFinder [database on the Internet]. ©1998–2013. 3,3′-Dimethylbenzidine dihydrochloride (612-82-8). PerkinElmer Inc. Available from: www.cambridgesoft.com/databases/login/?serviceid=128

Chemexper [database on the Internet]. 2012. Direct Red 2 (992-59-6). [cited 2012 Nov]. Available from: www.chemexper.net/specification_d/chemicals/supplier/cas/Benzopurpurin%204B.asp

ChemicalBook [database on the Internet]. 2008a. Direct Blue 14 (72-57-1) basic information. [cited 2012 Aug 2012]. Available from: www.chemicalbook.com/ProductChemicalPropertiesCB8315001_EN.htm

ChemicalBook [database on the Internet]. 2008b. Direct Black 38 (1937-37-7) basic information. [cited 2012 Aug 2012]. Available from: www.chemicalbook.com/ProductChemicalPropertiesCB0745257_EN.htm

ChemicalBook [database on the Internet]. 2008c. Direct Red 28 (573-58-0) basic information. [cited 2012 Aug]. Available from: www.chemicalbook.com/ProductChemicalPropertiesCB1261000_EN.htm

ChemicalBook [database on the Internet]. 2008d. Direct Brown 95 (16071-86-7) basic information. [cited 2012 Aug]. Available from: www.chemicalbook.com/ProductChemicalPropertiesCB6875055_EN.htm

ChemicalBook [database on the Internet]. 2008e. Direct Blue 15 (2429-74-5) basic information. [cited 2012 Aug]. Available from: www.chemicalbook.com/ProductChemicalPropertiesCB8315001_EN.htm

ChemicalBook [database on the Internet]. 2008f. Benzoazurine (2429-71-2) basic information. [cited 2012 Aug]. Available from: www.chemicalbook.com/ProductChemicalPropertiesCB0298223_EN.htm

ChemicalBook [database on the Internet]. 2008g. Azo blue (6420-06-0) basic information. [cited 2012 Aug]. Available from: www.chemicalbook.com/ProductChemicalPropertiesCB4176686_EN.htm

ChemicalBook [database on the Internet]. 2008h. Disodium 3-[[4′-[(6-amino-1-hydroxy-3-sulphonato-2-naphthyl) (6449-35-0) basic information. [cited 2012 Aug]. Available from: www.chemicalbook.com/ProductChemicalPropertiesCB1929496_EN.htm

ChemicalBook [database on the Internet]. 2008i. Tetrazolium Blue Chloride. (1871-22-3) basic information. [cited 2012 Aug]. Available from: www.chemicalbook.com/Search_EN.aspx?keyword=1871-22-3

ChemicalBook [database on the Internet]. 2008j. Nitrotetrazolium blue chloride (298-83-9) basic information. [cited 2012 Aug]. Available from: www.chemicalbook.com/Search_EN.aspx?keyword=298-83-9

ChemicalBook [database on the Internet]. 2008k. 3,3'-Dimethoxybenzidine (119-90-4) basic information. [cited 2012 Aug]. Available from: www.chemicalbook.com/ChemicalProductProperty_EN_CB7385595.htm

ChemicalBook [database on the Internet]. 2008l. 3,3'-Dimethyl-4,4'-biphenylene diisocyanate (91-97-4) basic information. [cited 2012 Aug]. Available from: www.chemicalbook.com/ProductChemicalPropertiesCB2485599_EN.htm#MSDSA

ChemicalLand21. 2010. Benzidine Yellow GR. [cited 2012]. Available from: www.chemicalland21.com/specialtychem/finechem/BENZIDINE%20YELLOW%20GR.htm

ChemIDplus [Internet chemicals search system]. 1993. Bethesda (MD): National Library of Medicine (US). Available from: http://chem.sis.nlm.nih.gov/chemidplus/

ChemSpider [online database]. ©2011. Cambridge (GB): Royal Society of Chemistry. Available from: www.chemspider.com/LiteratureSearch.aspx

Chen S, Nyman MC. 2009. Influence of pH on sediment-associated sorption of benzidine. Environ Toxicol Chem 28(9):1834–1841.

Chen SC, Kao CM, Huang MH, Shih MK, Chen YL, Huang SP, Liu TZ. 2003. Assessment of genotoxicity of benzidine and its structural analogues to human lymphocytes using comet assay. Toxicol Sci 72:283–288.

[CHRIP] Chemical Risk Information Platform [database on the Internet]. ©2002-2012. Tokyo (JP): National Institute of Technology and Evaluation, Chemical Management Centre. [cited 2011 Jan]. Available from: www.safe.nite.go.jp/english/db.html

Chung KT, Fulk GE, Andrews AW. 1981. Mutagenicity testing of some commonly used dyes. Appl Environ Microbiol 42(4):641–648.

Chung KT, Stevens SEJR, Cerniglia CE. 1992. The reduction of azo dyes by the intestinal microflora. Crit Rev Microbiol 18(3):175–190.

Chung KT, Chen SC, Wong TY, Wei CI. 1998. Effects of benzidine and benzidine analogues on growth of bacteria including *Azotobacter vinelandii*. Environ Toxicol Chem 17(2):271–275.

Chung K, Chen S, Wong T, Li Y, Wei C, Chou M. 2000. Mutagenicity studies of benzidine and its analogs: structure–activity relationships. Toxicol Sci 56(2):351–356.

Chung KT, Chen SC, Claxton LD. 2006. Review of the *Salmonella typhimurium* mutagenicity of benzidine, benzidine analogues, and benzidine-based dyes. Mutat Res 612(1):58–76.Cihak R. 1979. Evaluation of benzidine by the micronucleus test. Mutat Res 67(4):383–384.

[CII] Colour Index International [database on the Internet]. 2011. 4th ed. online [Internet]. Research Triangle Park (NC): Society of Dyers and Colourists and American Association of Textile Chemists and Colorists. Available from: www.colour-index.com

Clariant. 2008. The New Clariant Textile Dyestuff World. 3rd ed [Internet]. Muttenz (CH): Clariant International AG. [cited 2008 Dec]. Available from: www.textiles.clariant.com/C12571C400485A16/vwLookupDownloads/NewDyestuffWorld.pdf/$FILE/NewDyestuffWorld.pdf

Colon D, Weber EJ, Baughman GL. 2002. Sediment-associated reactions of aromatic amines. 2, QSAR development. Environ Sci Technol 36:2443–2450.

[CPDB] Carcinogenic Potency Database [database on the Internet]. Berkeley (CA): University of California. [cited 2012 Sep]. Available from: http://potency.berkeley.edu/

[CPOPs] Canadian Persistent Organic Pollutants Profiler Model. 2008. Version 1.1.18. Gatineau (QC): Environment Canada, Existing Substances Division; Bourgas (BG): Bourgas Prof. Assen Zlatarov University, Laboratory of Mathematical Chemistry. [Model developed based on Mekenyan et al. 2005].

Crechem Technologies Inc. 1998. Canadian wet processing textile mills: industry categorization, process description, water consumption, chemical usage and wastewater generation. Unpublished report prepared for Environment Canada and National Research Council of Canada (February 10, 1998; updated November 25, 1999).

[Crechem] Crechem Technologies Inc. 2005. Fate of wastewater sludge. Report prepared for Existing Substances Division, Environment Canada, Gatineau, QC, June. Ottawa (ON): Crechem.

[Danish EPA] Danish Environmental Protection Agency. 2012. Annex XV report: Proposal for a restriction. Substance name(s): Chromium (VI) compounds [Internet]. Available from: http://echa.europa.eu/documents/10162/4d88d444-4b8b-48ab-9c11-6e74819e047c

Debnath AK, Hansch C. 1992. Structure–activity relationship of genotoxic polycyclic aromatic nitro compounds: further evidence for the importance of hydrophobicity and molecular orbital energies in genetic toxicity. Environ Mol Mutagen 20(2):140–144.

De France BF, Carter MH, Josephy PD. 1986. Comparative metabolism and mutagenicity of azo and hydrazone dyes in the Ames test. Food Chem Toxicol 24(2):165–169.

Dehn WM. 1917. Comparative solubilities in water, in pyridine and in aqueous pyridine. J Am Chem Soc 39(7):1399–1404.

Dellarco VL, Prival MJ. 1989. Mutagenicity of nitro compounds in *Salmonella typhimurium* in the presence of flavin mononucleotide in a preincubation assay. Environ Mol Mutagen 13(2):116–127.

DEREK Nexus [Toxicity Prediction Module]. 2010. Version 2.0.3. Leeds (GB): Lhasa Ltd. [cited 2012 Aug 23]. Available from: https://www.lhasalimited.org/derek_nexus/ [restricted access] [DFG] Deutsche Forschungsgemeinschaft. 1993. 3,3′-Dimethoxybenzidine. In: Occupational toxicants: critical data evaluation for MAK values and classification of carcinogens. Weinheim (DE): VCH Publishers. p. 139.

Dillon D, Combes R, Zeiger E. 1994. Activation by caecal reduction of the azo dye D & C red no. 9 to a bacterial mutagen. Mutagenesis 9(4):295–299.

Douglas MT, Chanter DO, Pell IB, Burney GM. 1986. A proposal for the reduction of animal numbers required for the acute toxicity to fish test (LC50 determination). Aquat Toxicol 8(4):243–249.

[DPD] Drug Product Database [database on the Internet]. 2012. Ottawa (ON): Health Canada. [cited 2012]. Available from: www.hc-sc.gc.ca/dhp-mps/prodpharma/databasdon/index-eng.php

Droste RL. 1997. Theory and practice of water and wastewater treatment. Hoboken (NJ): John Wiley & Sons Inc.

[DS TOPKAT] Discovery Studio TOxicity Prediction by Komputer Assisted Technology [Prediction Module]. ©2005–2009. Version 2.5. San Diego (CA): Accelrys Software Inc. Available from: www.accelrys.com/products/

[ECHA] European Chemicals Agency. 2010. Guidance on information requirements and chemical safety assessment. Chapter R.16: Environmental exposure estimation, Version 2. Helsinki (FI): European Chemicals Agency. Available from: www.echa.europa.eu/documents/10162/13632/information_requirements_r16_en.pdf

[ECHA] European Chemicals Agency. 2012. Registered Substances database. Search results for various CAS RNs Helsinki (FI): ECHA. [cited 2012 Jan–Nov]. Available from: www.echa.europa.eu/information-on-chemicals/registered-substances

[ECJRC] European Commission Joint Research Centre. European Union risk assessment report: CAS: 101-77-9: 4,4′-methylenedianiline [Internet]. 2001. Luxembourg: Office for Official Publications of the European Communities. Report No:

EUR 19727 EN. Available from: http://esis.jrc.ec.europa.eu/doc/risk_assessment/REPORT/mdareport008.pdf

[ECOSAR] Ecological Structure Activity Relationships [Internet]. 2011. Version 1.10. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

[ECS] European Committee for Standardization. 2012. BS EN 14362-1:2012: Textiles—Methods for the determination of certain aromatic amines derived from azo colorants—Part 1: Detection of the use of certain azo colorants accessible with and without extracting the fibers. Brussels (BE): ECS.

EI DuPont de Nemours and Co Inc. 1978. Mutagenic activity in the *Salmonella*/microsome assay (CAS no. 2429-74-5). Case No: OTS84003A; Microfiche No.: 215029.

Elliott J, Gregory AR. 1980. Mutagenicity of a series of benzidine congener based dyes. Vet Hum Toxicol 22(6):413–417.

Environment Canada. 2006a. Data for Batch 6 substances collected under the Canadian Environmental Protection Act, 1999, Section 71: *Notice with respect to certain Batch 6 Challenge substances.* Data compiled by: Environment Canada, Program Development and Engagement Division.

Environment Canada. 2006b. Data for selected substances collected under the Canadian Environmental Protection Act, 1999, Section 71: *Notice with respect to selected substances identified as priority for action.* Data compiled by: Environment Canada, Program Development and Engagement Division.

Environment Canada. 2007. Guidance for conducting ecological assessments under CEPA, 1999: Science resource technical series, technical guidance module: QSARs. Reviewed draft working document. Gatineau (QC): Environment Canada, Ecological Assessment Division.

Environment Canada. 2008. Data for Batch 6 substances collected under the Canadian Environmental Protection Act, 1999, Section 71: *Notice with respect to certain Batch 6 Challenge substances.* Data compiled by: Environment Canada, Program Development and Engagement Division.

Environment Canada. 2009. Data relating to the Domestic Substance List (DSL) 2008, collected under CEPA 1999. Based on Reporting for the Domestic Substances List. Data prepared by: Environment Canada.

Environment Canada. 2012. Data for certain aromatic amines and aromatic azo- and benzidine-based substances collected under the Canadian Environmental Protection Act, 1999, Section 71: *Notice with respect to certain aromatic amines and aromatic azo- and benzidine-based substances.* Data compiled by: Environment Canada, Program Development and Engagement Division.

Environment Canada, Health Canada. 2007. Chemical substances: Categorization [Internet]. Ottawa (ON): Government of Canada. [updated 2007 April 20; cited 2014 June 10]. Available from: http://www.chemicalsubstanceschimiques.gc.ca/approach-approche/categor-eng.php

Environment Canada, Health Canada. 2009. Screening assessment for the Challenge: 7-Naphthalenedisulfonic acid, 4-amino-3-[[4′-[(2,4-diaminophenyl)azo][1,1′-biphenyl]-4-yl]azo]-5-hydroxy-6-(phenylazo)-, disodium salt (Direct Black 38): Chemical Abstracts Service Registry Number 1937-37-7 [Internet]. Ottawa (ON): Environment Canada; Health Canada. [cited 2012 Feb 20]. Available from: www.ec.gc.ca/ese-ees/default.asp?lang=En&n=7C287737-1

Environment Canada, Health Canada. 2011. Screening Assessment for the Challenge: 2,7-Naphthalenedisulfonic acid, 3-[[2,2′-dimethyl-4′-[[4-[[(4-methylphenyl)sulfonyl]oxy]phenyl]azo][1,1′-biphenyl]-4-yl]azo]-4-hydroxy-, disodium salt (Acid Red 111): Chemical Abstracts Service Registry Number 6358-57-2 [Internet]. [cited 2012 Feb 28]. Ottawa (ON): Environment Canada; Health Canada. Available from: www.ec.gc.ca/ese-ees/default.asp?lang=En&n=A5B7D1E8-1

Environment Canada, Health Canada. 2013. The Chemicals Management Plan Substance Grouping Initiative. Subgrouping Approach and Background Information for the Screening Assessment of Aromatic Azo and Benzidine-Based Substances. May 2013. Environment Canada, Health Canada. Available upon request.

Environment Canada, Health Canada. 2014. Rapid Screening of Substances from Phase One of the Domestic Substances List Inventory Update. March 2014. Environment Canada, Health Canada. Available from: http://www.ec.gc.ca/ese-ees/7340E1B7-1809-4564-8C49-F05875D511CB/FSAR_RSII_EN.pdf

[EPI Suite] Estimation Programs Interface Suite for Microsoft Windows [Estimation Model]. 2012. Version 4.1. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

[EQC] Equilibrium Criterion Model. 2003. Version 2.02. Peterborough (ON): Trent University, Canadian Centre for Environmental Modelling and Chemistry. Available from: www.trentu.ca/academic/aminss/envmodel/models/EQC2.html

Ericson JW. 1977. Applicability of ATP measurements for the determination of dye toxicity in short term algal assays. In: Proceedings of the Bi-Annual ATP Methodology Symposium. San Diego (CA): SAI Technology Co. p. 415–439.

[ESIS] European Chemical Substances Information System [database on the Internet]. ©1995–2012. Joint Research Centre (JRC). [cited 2012]. Available from: http://esis.jrc.ec.europa.eu/

[ETAD] Ecological and Toxicological Association of Dyes and Organic Pigment Manufacturers. 1983a. Status report: On ETAD studies of relevance within the context of the EPA decision not require the effects testing of benzidine-, *o*-tolidine, and *o*-dianisidine-based dyes. Appendix II, report on *Salmonella*/microsome mutation test. Report No.: Doc #40-8229019 6459-94-5. p. 1–52.

[ETAD] Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers. 1983b. Final report on extractability of dyestuffs from textiles. Project A 4007.

[ETAD] Ecological and Toxicological Association of Dyes and Organic Pigment Manufacturers. 1985. Results from the *Salmonella*/microsome mutagenicity assay, cover letter dated 10/29/85. Report No.: T 2015-B.

[ETAD] Ecological and Toxicological Association of Dyes and Organic Pigment Manufacturers. 1986. C.I. Acid Red 114: Study of the mutagenic potential in strains of *Salmonella typhimurium* (Ames test) and *Escherichia coli* with cover letter dated 06/10/86. Report No.: Doc #868600075.

[ETAD] Ecological and Toxicological Association of Dyes and Organic Pigment Manufacturers. 1988. Executive summary on the ETAD project T 2015 toxicological testing of major colorants, with attachments and cover letter dated 06/03/88. Report No.: TSCA OTS0000621, Doc #FYI-AX-0688-0621.

[ETAD] Ecological and Toxicological of Dyes and Organic Pigments Manufacturers. 1989. Various individual ETAD internal reports of unpublished studies by ETAD member companies. [cited in Jung et al 1992]

[ETAD] Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers. 1994. *In vitro* absorption of various dyes through human and pig epidermis. Project T 2030, Part 1.

[ETAD] Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers. 1995a. *In vitro* absorption of two disperse dyes from synthetic perspiration and five formulations. Project T 2030, Part 2.

[ETAD] Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers. 1995b. Health & environmental information on dyes used in Canada. An overview to assist in the implementation of the *New Substances Notification Regulations* under the *Canadian Environmental Protection Act*. Prepared by the ETAD Canadian Affiliates. Report No.: 7/21/95.

European Committee for Standardization, EN14362-1:2003, Textiles - Methods for the determination of certain aromatic amines derived from azo colorants - Part 1: Detection of the use of certain azo colorants accessible without extraction. 2003. Brussels.

European Committee for Standardization, EN14362-2:2003, Textiles - Methods for the determination of certain aromatic amines derived from azo colorants - Part 2: Detection of the use of certain azo colorants accessible by extracting the fibers. 2003. Brussels.

[EU] European Union. 2001. Directive 2001/95/EC of the European Parliament and of the Council of 3 December 2001 on general product safety [Internet]. Off J Eur L 11:4–17. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2002:011:0004:0017:en:PDF

[EU] European Union. 2006. Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC [Internet]. Off J Eur Union L 396:1–849. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=oj:l:2006:396:0001:0849:en:pdf

Field FE, Roberts G, Hallowes RC, Palmer AK, Williams KE, Lloyd JB. 1977. Trypan blue: identification and teratogenic and oncogenic activities of its coloured constituents. Chem Biol Interact 16(1):69–88.

Fitzgerald GP, Gerloff GC, Skoog F. 1952. Studies on chemicals with selective toxicity to blue-green algae. Sewage Ind Wastes 24(7):888–896.

Ford RJ, Becker FF. 1982. The characterization of trypan blue–induced tumors in Wistar rats. Am J Pathol 106(3):326–331.

Freeman HS, Esancy JF, Esancy MK, Mills KP, Whaley WM, Dabney BJ. 1987. An approach to the design of non-mutagenic azo dyes. 1. The identification of non-mutagenic precursors and potential metabolites. Dyes Pigm 8(6):417–430.

Freeman HS. 2011. Assessment report to Health Canada: Peer review of the Aromatic Azo and Benzidine-Based Substances Grouping approach. Raleigh (NC): North Carolina State University, Polymer and Color Chemistry Program.

Freitag D, Ballhorn L, Geyer H, Korte F. 1985. Environmental hazard profile for organic chemicals: an experimental method for the assessment of the behaviour of organic chemicals in the ecosphere by means of simple laboratory tests with ^{14}C-labelled chemicals. Chemosphere 14:1589–1616.

Galloway SM, Bloom AD, Resnick M, Margolin BH, Nakamura F, Archer P, Zeiger E. 1985. Development of a standard protocol for *in vitro* cytogenetic testing with Chinese hamster ovary cells: comparison of results for 22 compounds in two laboratories. Environ Mutagen 7:1–51.

Galloway SM, Armstrong MJ, Reuben C, Colman S, Brown B, Cannon C, Bloom AD, Nakamura F, Ahmed M, Duk S, Rimpo J, Margolin BH, Resnick MA, Anderson B, Zeiger E. 1987. Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. Environ Mol Mutagen 10(Suppl 10):1–35.

Garner RC, Nutman CA. 1977. Testing of some azo dyes and their reduction products for mutagenicity using *Salmonella typhimurium* TA1538. Mutat Res 44:9–19.

Ge X, Wexler AS, Clegg SL. 2011. Atmospheric amines—Part 1: A review. Atmos Environ 45(3):524–546.

Gillman J, Gillman T. 1952. The pathogenesis of experimentally produced lymphomata in rats (including Hodgkin's-like sarcoma). Cancer 5(4):792–846.

Gillman T, Kinns AM, Hallowes RC, Lloyd JB. 1973. Malignant lymphoreticular tumors induced by trypan blue and transplanted in inbred rats. J Natl Cancer Inst 50:1179–1193. [cited in IARC 1975].

Gobas FAPC, Kelly BC, Arnot JA. 2003. Quantitative structure activity relationships for predicting the bioaccumulation of POPs in terrestrial food-webs. QSAR Comb Sci 22:329–336.

Gorecka-Turska D, Mekler U, Gorski T. 1983. Sister chromatid exchanges in Balb-C mouse bone-marrow in response to carcinogenic and noncarcinogenic products and intermediates from dye manufacture. Bromatol Chem Toksykol 16:37–42. [cited in DFG 1993].

Green FJ. 1990. The Sigma-Aldrich handbook for dyes, stains and indicators. Milwaukee (WI): Aldrich Chemical Co. p. 513. Cited in PhysProp.

Gregory AR, Elliott J, Kluge P. 1981. Ames testing of Direct Black 38 parallels carcinogenicity testing. J Appl Toxicol 1(6):308–313.

Griswold DP Jr, Casey AE, Weisburger EK, Weisburger JH. 1968. The carcinogenicity of multiple intragastric doses of aromatic and heterocyclic nitro or amino derivatives in young female Sprague-Dawley rats. Cancer Res 28(5):924–933. [cited in IARC 2010d].

Hadidian Z, Fredrickson TN, Weisburger EK, Weisburger JH, Glass RM, Mantel N. 1968. Tests for chemical carcinogens. Report on the activity of derivatives of aromatic amines, nitrosamines, quinolines, nitroalkanes, amides, epoxides, aziridines, and purine antimetabolites. J Natl Cancer Inst 41(4):985–1036. [cited in IARC 1974a, 2010b].

Hamann HJ, Richard D, Robert N, Knuppe C. 2000. 13-week oral toxicity (gavage) study in rat with D&C Orange 4 (C.I. 15510). Itingen (CH): RCC Ltd. RCC Project No. 736312, Test Report.

Hansch C, Hoekman D, Leo A, Zhang L, Li P. 1995. The expanding role of quantitative structure–activity relationships (QSAR) in toxicology. Toxicol Lett 79(1–3):45–53.

Hartman CP, Fulk GE, Andrews AW. 1978. Azo reduction of trypan blue to a known carcinogen by a cell-free extract of a human intestinal anaerobe. Mutat Res 58(2–3):125–132.

Hawley GG. 1981. The condensed chemical dictionary. 10th ed. New York (NY): Van Nostrand Reinhold Co. p. 1029.

Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E. 1983. *Salmonella* mutagenicity test results for 250 chemicals. Environ Mutagen 5(Suppl 1):3–142.

Health Canada. 1995. Investigating human exposure to contaminants in the environment: a handbook for exposure calculations. Ottawa (ON): Health Canada, Health Protection Branch, Great Lakes Health Effects Program.

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

Health Canada. 2011a. The Cosmetic Ingredient Hotlist March 2011 [Internet]. Ottawa (ON): Health Canada, Consumer Product Safety. [cited 2012 September]. Available from: www.hc-sc.gc.ca/cps-spc/cosmet-person/indust/hot-list-critique/hotlist-liste-eng.php

Health Canada. 2011b. Follow-up report on a PSL substance: Aniline. Chemical Abstracts Service Registry Number 62-53-3. Available from: www.chemicalsubstanceschimiques.gc.ca/plan/approach-approche/aniline-eng.php

Health Canada. 2012. List of permitted colouring agents (lists of permitted food additives). [cited 2012 September]. Available from: www.hc-sc.gc.ca/fn-an/securit/addit/list/3-colour-color-eng.php

Health Canada. 2013. Determination of total aromatic amines derived from azo dyes in textiles and leathers by LC-MS-MS: Survey 2012–-2013 [Draft]. Project No# 2012-1459. Ottawa (ON): Health Canada, Product Safety Laboratory (CAN). Available upon request.

[HENRYWIN] Henry's Law Constant Program for Microsoft Windows [Estimation Model]. 2011. Version 3.20. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

Hinks D, Freeman HS, Nakpathom M, Sokolowska J. 2000. Synthesis and evaluation of organic pigments and intermediates. 1. Nonmutagenic benzidine analogs. Dyes Pigments 44(3):199–207.

[HSDB] Hazardous Substances Data Bank [database on the Internet]. 1983– . Bethesda (MD): National Library of Medicine (US). [cited 2012]. Available from: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB

Hulzebos EM, Adema DMM, Dirven-Van Breemen EM, Henzen L, Van Dis WA, Herbold HA, Hoekstra JA, Baerselman R, Van Gestel CAM. 1993. Phytotoxicity studies with *Lactuca sativa* in soil and nutrient solution. Environ Toxicol Chem 12(6):1079–1094.

Hunger K, editor. 2003. Industrial dyes: chemistry, properties, applications. Weinheim (DE): Wiley-VCH Verlag GmbH & Co. KGaA.

Hunger K. 2005. Toxicology and toxicological testing of colorants. Rev Prog Color 35:76–89.

[HYDROWIN] Hydrolysis Rates Program for Microsoft Windows [Estimation Model]. 2010. Version 2.00. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1972. 3,3′-Dimethylbenzidine (*o*-tolidine). In: Some inorganic substances, chlorinated hydrocarbons, aromatic amines, *N*-nitroso compounds and natural products. IARC Monogr Eval Carcinog Risk Hum 1:87–91.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1974a. 3,3′-Dimethoxybenzidine. In: Some aromatic amines, hydrazine and related substances, *N*-nitroso compounds and miscellaneous alkylating agents. IARC Monogr Eval Carcinog Risk Hum 4:41–47.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1974b. 3,3′-Dichlorobenzidine. In: Some aromatic amines, hydrazine and related substances, *N*-nitroso compounds and miscellaneous alkylating agents. IARC Monogr Eval Carcinog Risk Hum 4:49–55.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1975. Trypan blue. In: Some aromatic azo compounds. IARC Monogr Eval Carcinog Risk Hum 8:267–278.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1982a. Direct Black 38. In: Some industrial chemicals and dyestuffs. IARC Monogr Eval Carcinog Risk Hum 29:295–310.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1982b. 3,3′-Dichlorobenzidine and its dihydrochloride. In: Some industrial chemicals and dyestuffs. IARC Monogr Eval Carcinog Risk Chem Hum 29:239–256.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1986. 3,3′-Dimethoxybenzidine-4,4′-diisocyanate. In: Some chemicals used in plastics and elastomers. IARC Monogr Eval Carcinog Risk Chem Hum 39:279–286.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1987a. 3,3′-Dimethoxybenzidine (group 2B). In: Overall evaluations of carcinogenicity: an updating of IARC monographs volumes 1–42. IARC Monogr Eval Carcinog Risk Chem Hum Suppl 7:198–199.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1987b. Overall evaluations of carcinogenicity. In: Overall evaluations of carcinogenicity: an updating of IARC monographs volumes 1–42. IARC Monogr Eval Carcinog Risk Chem Hum Suppl 7:37–74.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1987c. *ortho*-Toluidine (group 2B). In: Overall evaluations of carcinogenicity: an updating of IARC monographs volumes 1–42. IARC Monogr Eval Carcinog Risk Chem Hum Suppl 7:362–363.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1993a. CI Direct Blue 15. In: Occupational exposures of hairdressers and barbers and personal use of hair colourants; some hair dyes, cosmetic colourants, industrial dyestuffs and aromatic amines. IARC Monogr Eval Carcinog Risk Chem Hum 57:235–245.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1993b. CI Acid Red 114. In: Occupational exposures of hairdressers and barbers and personal use of hair colourants; some hair dyes, cosmetic colourants, industrial dyestuffs and aromatic amines. IARC Monogr Eval Carcinog Risk Chem Hum 57:247–257.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1997. Summary of data reported and evaluation. In: Occupational exposures of hairdressers and barbers and personal use of hair colourants; some hair dyes, cosmetic colourants, industrial dyestuffs and aromatic amines. IARC Monogr Eval Carcinog Risk Chem Hum 57:30–31.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 2010a. Dyes metabolized to benzidine. In: Some aromatic amines, organic dyes, and related exposures. IARC Monogr Eval Carcinog Risk Chem Hum 99:263–296.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 2010b. 3,3′-Dimethoxybenzidine. In: Some aromatic amines, organic dyes, and related exposures. IARC Monogr Eval Carcinog Risk Chem Hum 99:210–213.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 2010c. 3,3′-Dichlorobenzidine and its dihydrochloride. In: Some aromatic amines, organic dyes, and related exposures. IARC Monogr Eval Carcinog Risk Chem Hum 99:206–210.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 2010d. 3,3′-Dimethylbenzidine. In: Some aromatic amines, organic dyes, and related exposures. IARC Monogr Eval Carcinog Risk Chem Hum 99:214–218.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 2010e. Some aromatic amines, organic dyes, and related exposures. IARC Monogr Eval Carcinog Risks Hum 99. Available from: http://monographs.iarc.fr/ENG/Monographs/vol99/mono99.pdf

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 2012a. Dyes metabolized to benzidine. In: Chemical Agents and Related Occupations.

A Review of Human Carcinogens. IARC Monogr Eval Carcinog Risk Chem Hum 100:65-72.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 2012b. Benzidine. In: Chemical Agents and Related Occupations. A Review of Human Carcinogens. IARC Monogr Eval Carcinog Risk Chem Hum 100:53-63.

[ICSC] International Chemical Safety Cards. 1998. ICSC 0960: *o*-Tolidine [Internet]. Available from: www.cdc.gov/niosh/ipcsneng/neng0960.html

Idaka E, Ogawa T, Yatome C, Horitsu H. 1985. Behaviour of activated sludge with dyes. Bull Environ Contam Toxicol 35(1):729–734.

[ILS] Integrated Laboratory Systems, Inc. 2011a. Final report: Assessment of mutagenicity of ten benzidine-based azo substances in the Ames mutagenicity assay using the Prival modification with and without FMN. Unpublished report prepared for Health Canada. Durham (NC): ILS. Study Report No.: C191-002.

[ILS] Integrated Laboratory Systems, Inc. 2011b. Final report: Assessment of mutagenicity of four benzidine-based azo substances in the Ames mutagenicity assay using the Prival modification with and without FMN. Unpublished report prepared for Health Canada. Durham (NC): ILS. Study Report No.: C191-004.

Industry Canada. 2012. Trade data online (TDO) [Internet]. [cited 2012 Aug 1]. Available from: www.ic.gc.ca/eic/site/tdo-dcd.nsf/eng/Home

Isik M, Sponza DT. 2004. Monitoring of toxicity and intermediates of C.I. Direct Black 38 azo dye through decolourization in an anaerobic/aerobic sequential reactor system. J Hazard Mater B114:29–39.

[JETOC] Japan Chemical Industry Ecology-Toxicology & Information Center. 1996. Mutagenicity test data of existing chemical substances. Based on the toxicity investigation of the industrial safety and health law. Compiled under the supervision of the Chemical Substance Investigation Division, Ministry of Labour, Japan. Tokyo (JP): JETOC. [cited in CCRIS 2012].

[JETOC] Japan Chemical Industry Ecology-Toxicology & Information Center. 2000. Mutagenicity test data of existing chemical substances. Based on the toxicity investigation system of the industrial safety and health law. Tests on dimethoxybenzidine (Suppl. 2). Compiled under the supervision of the Chemical Substance Investigation Division, Ministry of Labour, Japan. Tokyo (JP): JETOC.

Joachim F, Decad GM. 1984. Induction of unscheduled DNA synthesis in primary rat hepatocytes by benzidine-congener-derived azo dyes in the *in vitro* and *in vivo*/*in vitro* assays. Mutat Res 136(2):147–152.

Joachim F, Burrell A, Andersen J. 1985. Mutagenicity of azo dyes in the *Salmonella*/microsome assay using *in vitro* and *in vivo* activation. Mutat Res 156(3):131–138.

Joneja M, Ungthavorn S. 1968. Chromosome aberrations in trypan blue induced teratogenesis in mice. Can J Genet Cytol 10(1):91–98.

Jung R, Steinle D, Anliker R. 1992. A compilation of genotoxicity and carcinogenicity data on aromatic aminosulphonic acids. Food Chem Toxicol 30(7):635–660.

Kanto Chemical Co., Inc. 2011. Catalogue of fine chemicals. Available from: www.kanto.co.jp/english/index.html

Kawakami T, Isama K, Nakashima H, Tsuchiya T, Matsuoka A. 2010. Analysis of primary aromatic amines originated from azo dyes in commercial textile products in Japan. J Environ Sci Health A 45(10):1281–1295.

Kawasaki M. 1980. Experiences with the test scheme under the Chemical Control Law of Japan: an approach to structure–activity correlations. Ecotoxicol Environ Saf 4:444–454.

Kelly BC, Ikonomou MG, Blair JD, Morin AE, Gobas FAPC. 2007. Food web–specific biomagnification of persistent organic pollutants. Science 317(236):236–239.

Kennelly JC, Hertzog PJ, Martin CN. 1982. The release of 4,4′-diaminobiphenyls from azodyes in the rat. Carcinogenesis 3(8):947–951.

Kennelly JC, Shaw A, Martin CN. 1984a. Reduction to benzidine is not necessary for the covalent binding of a benzidine azodye to rat liver DNA. Toxicology 32(4):315–324.

Kennelly JC, Stanton CA, Martin CN. 1984b. The effect of acetyl-CoA supplementation on the mutagenicity of benzidines in the Ames assay. Mutat Res 137(1):39–45.

[Kirk-Othmer] Kirk-Othmer Encyclopedia of Chemical Technology. 1981. John Wiley & Sons, Inc.

[Kirk-Othmer] Kirk-Othmer Encyclopedia of Chemical Technology. 2010. John Wiley & Sons, Inc. Available from: http://onlinelibrary.wiley.com/book/10.1002/0471238961/

[KOAWIN] Octanol Air Partition Coefficient Program for Microsoft Windows [Estimation Model]. 2010. Version 1.10. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

[KOCWIN] Organ Carbon Partition Coefficient Program for Windows [Estimation Model]. 2010. Version 2.00. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

Kornbrust DJ, Barfknecht TR. 1984. Comparison of 7 azo dyes and their azo reduction products in the rat and hamster hepatocyte primary culture/DNA-repair assays. Mutat Res 136(3):255–266.

Kornbrust D, Barfknecht T. 1985. Testing of 24 food, drug, cosmetic, and fabric dyes in the *in vitro* and the *in vivo*/*in vitro* rat hepatocytes primary culture/DNA-repair assay. Environ Mutagen 7:101–120.

Kosaka H, Nakamura S. 1990. Genotoxicity of synthetic dyes in umu test using *Salmonella typhimurium* TA1535/pSK1002 (1). Results of examination for acid dyes, direct dyes, disperse dyes and reactive dyes. Sangyo Igaku 32(2):89–104.

[KOWWIN] Octanol–Water Partition Coefficient Program for Microsoft Windows [Estimation Model]. 2010. Version 1.68. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

Krishna G, Xu J, Nath J. 1986. Comparative mutagenicity studies of azo dyes and their reduction products in *Salmonella typhimurium*. J Toxicol Environ Health 18(1):111–119.

Kubota T, Ezumi K. 1980. Simultaneous determination of acid dissociation constants and true partition coefficients by analysis of the apparent partition coefficients. II. Dibasic acid and diacidic base. Chem Pharm Bull 28:3673–3678.

Kuhn R, Pattard M, Pernak K-D, Winter A. 1989. Results of the harmful effects of pollutants to *Daphnia magna* in 21 day reproduction test. Water Res 23(4):501–510.

Levine RA, Oller WL, Nony CR, Bowman MC. 1982. Benzidine-congener-based azo dyes: assays for purity and residues in feces from dosed rats. J Anal Toxicol 6(4):157–163.

Lewis RJ Sr, editor. 1997. Hawley's condensed chemical dictionary. New York (NY): John Wiley & Sons. p. 350.

Lide DR, editor. 2002. CRC handbook of chemistry and physics. 83rd ed. Boca Raton (FL): CRC Press.

Lin GHY, Solodar WE. 1988. Structure–activity relationship studies on the mutagenicity of some azo dyes in the *Salmonella*/microsome assay. Mutagenesis 3(4):311–315.

Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 46:3–26.

Little LW, Lamb JC III. 1972. Acute toxicity of 46 selected dyes to the fathead minnow, *Pimephales promelas*. Chapel Hill (NC): University of North Carolina, Department of Environmental Sciences and Engineering, School of Public Health, UNC Wastewater Research Center. Prepared for Ecology Committee, American Dye Manufacturers, Inc., September. 126 p.

Little LW, Lamb JC III. 1973. Acute toxicity of 46 selected dyes to the fathead minnow, *Pimephales promelas*. In: Dyes and the environment—Reports on selected dyes and their effects. Vol. 1. American Dye Manufacturers Institute, Inc. 130 p.

Little LW, Lamb JC III. 1974. Acute toxicity of selected commercial dyes to the fathead minnow and evaluation of biological treatment for reduction of toxicity. In: Proceedings of the 29th Industrial Waste Conference, Purdue University, West Lafayette, IN. p. 524–534.

[LNHPD] Licensed Natural Health Products Database [database on the Internet]. 2008. Ottawa (ON): Health Canada. [cited 2011]. Available from: http://webprod3.hc-sc.gc.ca/lnhpd-bdpsnh/index-eng.jsp

Longstaff E, McGregor DB, Harris WJ, Robertson JA, Poole A. 1984. A comparison of the predictive values of the *Salmonella*/microsome mutation and BHK21 cell transformation assays in relation to dyestuffs and similar materials. Dyes Pigments 5:65–82.

Lu PY, Metcalf RL, Plummer N, Mandel D. 1977. The environmental fate of three carcinogens: benzo(*a*)pyrene, benzidine and vinyl chloride evaluated in laboratory model ecosystems. Arch Environ Contam Toxicol 6(2–3):129–142 [cited in Canada 1993a].

Lynn RK, Donielson DW, Ilias AM, Kennish JM, Wong K, Matthews HB. 1980. Metabolism of bisazobiphenyl dyes derived from benzidine, 3,3′-dimethylbenzidine or 3,3′-dimethoxybenzidine to carcinogenic aromatic amines in the dog and rat. Toxicol Appl Pharmacol 56(2):248–258.

MacPhee C, Ruelle R. 1969. Lethal effects of 1888 chemicals upon four species of fish from western North America. Moscow (RU): Forest, Wildlife, and Range Experiment Station.

Makena PS, Chung K. 2007. Evidence that 4-aminobiphenyl, benzidine, and benzidine congeners produce genotoxicity through reactive oxygen species. Environ Mol Mutagen 48(5):404–413.

Marchisio MA, Dubini M, Serra G. 1976. Excretion of ^{35}S tobias acid (2 naphthylamino, 1 sulphonic acid) by the rat after oral and intravenous administration. Br J Ind Med 33(4):269–271.

Marmion DM. 1991. Handbook of U.S. colorants: foods, drugs, and cosmetics and medical devices. New York (NY): John Wiley & Sons, Inc. [cited in test plan for C.I. Acid Yellow 23 Submitted to the EPA under the HPV Challenge Program by: The International Association of Color Manufacturers/HPV Committee]

Martelli A, Robbiano L, Carrozzino R, Puglia CP, Mattioli F, Angiola M, Brambilla G. 2000. DNA damage induced by 3,3′-dimethoxybenzidine in liver and urinary bladder cells of rats and humans. Toxicol Sci 53(1):71–76.

Martin CN, Kennelly JC. 1981. Rat liver microsomal azoreductase activity on four azo dyes derived from benzidine, 3,3′-dimethylbenzidine or 3,3′-dimethoxybenzidine. Carcinogenesis 2(4):307–312.

Martin CN, McDermid AC, Garner RC. 1978. Testing of known carcinogens and noncarcinogens for their ability to induce unscheduled DNA synthesis in Hela cells. Cancer Res 38(8):2621–2627.

Matthews EJ, Spalding JW, Tennant RW. 1993. Transformation of BALB/c-3T3 cells: IV. Rank-ordered potency of 24 chemical responses detected in a sensitive new assay procedure. Environ Health Perspect 101(Suppl 2):319–345.

McCann J, Choi E, Yamasaki E, Ames BN. 1975. Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals. Proc Natl Acad Sci USA 72(12):5135–5139.

McConlogue L, Katzen AL, Andersen JJ, Burrell AD. 1980. The effect of aromatic amines of sister chromatid exchanges in Chinese hamster ovary cells. In: Proceedings of the 11th Annual Meeting of the Environmental Mutagen Society, March 16–19, 1980, Nashville, TN. Nashville (TN): Environmental Mutagen Society. p. 95.

McGirr LG, O'Brien PJ. 1987. Glutathione conjugate formation without *N*-demethylation during the peroxidase catalyzed *N*-oxidation of *N*,*N′*,*N*,*N′*-tetramethylbenzidine. Chem-Biol Interact 61(1):61–74.

Mekenyan OG, Dimitrov SD, Pavlov TS, Veith GD. 2004. A systems approach to simulating metabolism in computational toxicology. I. The TIMES heuristic modelling framework. Curr Pharm Design 10(11):1273–1293.

Mekenyan G, Dimitrov SD, Pavlov TS, Veith GD. 2005. POPs: a QSAR system for creating PBT profiles of chemicals and their metabolites. SAR QSAR Environ Res 16(1–2):103–133.

Merck Index. 1989. The Merck index: an encyclopedia of chemicals, drugs, and biologicals. Whitehouse Station (NJ): Merck Research Laboratories.

Merck Index. 2001. The Merck index: an encyclopedia of chemicals, drugs, and biologicals. 13th ed. Whitehouse Station (NJ): Merck & Co., Inc.

Merck Index. 2006. The Merck index: an encyclopedia of chemicals, drugs, and biologicals. Whitehouse Station (NJ): Merck Research Laboratories.

Merck Index. 2011. The Merck index: an encyclopedia of chemicals, drugs, and biologicals. Whitehouse Station (NJ): Merck Research Laboratories.

Messerly EA, Fekete JE, Wade DR, Sinsheimer JE. 1987. Structure–mutagenicity relationships of benzidine analogues. Environ Mol Mutagen 10(3):263–274.

Meylan WM, Howard PH. 1991. Bond contribution method for estimating Henry's law constants. Environ Toxicol Chem 10:1283–1291.

Meylan WM, Howard PH. 1993. Computer estimation of the atmospheric gas-phase reaction rate of organic compounds with hydroxyl radicals and ozone. Chemosphere 26(12):2293–2299.

Meylan W, Howard P, Boethling R. 1996. Improved method for estimating water solubility from octanol/water partition coefficient. Environ Toxicol Chem 15(2):100–106.

Michigan Institute of Laundering and Dry Cleaning. 2012. Consumer Guide to Clothing Care, Garment Life Expectancy (Depreciation). Available from: www.mildmi.org/facts_clothing_care.php

Mirsalis J, Tyson K, Beck J, Loh E, Steinmetz K, Contreras C, Austere L, Martin S, Spalding J. 1983. Induction of unscheduled DNA synthesis (UDS) in hepatocytes following *in vitro* and *in vivo* treatment. Environ Mutagen 5:482 (Abstract No. Ef-5).

Mitchell AD, Rudd CJ, Caspary WJ. 1988. Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: intralaboratory results for sixty-three coded chemicals tested at SRI International. Environ Mol Mutagen 12(Suppl 13):37–101.

[MITI] Ministry of International Trade and Industry (JP), Basic Industries Bureau, Chemical Products Safety Division. 1992. Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan. Tokyo (JP): Japan Chemical Industry Ecology-Toxicology & Information Center.

[MITI] Ministry of International Trade and Industry (JP), Basic Industries Bureau, Chemical Products Safety Division. 2000. Fish accumulation test result. Tokyo (JP): Japan Chemical Industry Ecology-Toxicology & Information Center.

Morgan DL, Bucher JR, Huff JE, Haseman JK, Eustis SL, Lemen JK, Ulland BM, Mennear JH. 1990. Multi-organ carcinogenicity of 3,3′-dimethoxybenzidine dihydrochloride given in drinking water to F344 rats. J Am Coll Toxicol 9(1):79–92.

Morgan DL, Jokinen MP, Haseman JK, Ulland BM, Parker GA, Lemen JK, Mennear JH. 1991. Carcinogenicity of 3,3′-dimethylbenzidine dihydrochloride given in drinking water to F344 rats. J Am Coll Toxicol 10(2):255–267.

Morgan DL, Dunnick JK, Goehl T, Jokinen MP, Matthews HB, Zeiger E, Mennear JH. 1994. Summary of the National Toxicology Program Benzidine Dye Initiative. Environ Health Perspect 102(Suppl 2):63–78.

Morita T, Asano N, Awogi T, Sasaki YF, Sato S, Shimada H, Sutou S, Suzuki T, Wakata A, Sofuni T, Hayashi M. 1997. Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B). The summary report of the 6th collaborative study by CSGMT/JEMS MMS. Collaborative Study of the Micronucleus Group Test. Mammalian Mutagenicity Study Group. Mutat Res 389(1):3–122. [published erratum appears in Mutat Res 1997, 391(3):259–267].

Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B, Zeiger E. 1986. *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. Environ Mutagen 8(Suppl 7):1–119.

[MPBPWIN] Melting Point Boiling Point Program for Microsoft Windows [Estimation Model]. 2010. Version 1.43. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

Myhr BC, Caspary WJ. 1988. Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: intralaboratory results for sixty-three coded chemicals tested at Litton Bionetics Inc. Environ Mol Mutagen 12(Suppl 13):103–194.

Nakamura S, Oda Y, Shimada T, Oki I, Sugimoto K. 1987. SOS-inducing activity of chemical carcinogens and mutagens in *Salmonella typhimurium* TA1535/pSK1002: examination with 151 chemicals. Mutat Res 192(4):239–246.

[NCI] National Chemical Inventories [database on a CD-ROM]. 2007. Issue 2. Columbus (OH): American Chemical Society. [cited 2012 Aug]. Available from: www.cas.org/products/other-cas-products/nci-on-cd

Neely WB, Blau GE, editors. 1985. Environmental exposure from chemicals. Vol. 1. Boca Raton (FL): CRC Press. p. 91–108.

[NHPID] Natural Health Products Ingredients Database [database on the Internet]. 2008. Ottawa (ON): Health Canada. [cited 2011]. Available from: http://webprod.hc-sc.gc.ca/nhpid-bdipsn/search-rechercheReq.do

[NIOSH] National Institute for Occupational Safety and Health (US). 2012. Pocket guide to chemical hazards. Atlanta (GA): Centers for Disease Control and Prevention. [cited 2012 Aug]. Available from: www.cdc.gov/niosh/npg/

Nony CR. 1979. Metabolism of azo dyes to potentially carcinogenic aromatic amines. (NCTR Technical Report for Experiment Number 196). Cincinnati (OH): National Institute for Occupational Safety and Health.

Nony CR, Bowman MC. 1980. Trace analysis of potentially carcinogenic metabolites of an azo dye and pigment in hamster and human urine as determined by two chromatographic procedures. J Chromatogr Sci 18(2):64–74.

Nony CR, Martin JL, Bowman MC. 1983. Metabolism of a dimethylbenzidine-based dye in rats and hamsters as determined by analysis of the urine for potentially carcinogenic aromatic amines. J Anal Toxicol 7(1):49–54.

[NTP] National Toxicology Program (US). 1979. Bioassay of 3,3′-dimethoxybenzidine-4,4′-diisocyanate for possible carcinogenicity (CAS No.91-93-0). Research Triangle Park (NC): US Department of Health and Human Services, National Toxicology Program. Report No.: 128.

[NTP] National Toxicology Program (US). 1983. National Toxicology Program studies of metabolism of azo dyes in rats. (National Center for Toxicological Research technical report (final) for experiment no. 6048 with cover letter dated 020783). Research Triangle Park (NC): US Department of Health and Human Services, National Toxicology Program.

[NTP] National Toxicology Program (US). 1990. Toxicology and carcinogenesis studies of 3,3′-dimethoxybenzidine dihydrochloride (CAS No. 20325-40-0) in F344/N rats (drinking water studies). Research Triangle Park (NC): US Department of Health and Human Services, National Toxicology Program. Report No.: 372. Available from: http://ntp.niehs.nih.gov//?objectid=0708E5AE-FCAD-C2FF-65EA7281D9BB61FA

[NTP] National Toxicology Program (US). 1991a. Toxicology and carcinogenesis studies of C.I. Acid Red 114 in F344/N rats (drinking water studies). Research Triangle Park (NC): US Department of Health and Human Services, National Toxicology Program. Report No.: 405. p. 241.

[NTP] National Toxicology Program (US). 1991b. Toxicology and carcinogenesis studies of 3,3′-dimethylbenzidine dihydrochloride (CAS No. 612-82-8) in F344/N rats (drinking water studies). Research Triangle Park (NC): US Department of Health and Human Services, National Toxicology Program. Report No.: 390. p. 3.

[NTP] National Toxicology Program (US). 1992. Toxicology and carcinogenesis studies of C.I. Direct Blue 15 (desalted industrial grade) (CAS No. 2429-74-5) in F344/N rats (drinking water studies). Research Triangle Park (NC): US Department of Health and Human Services, National Toxicology Program. Report No.: NTP TR 397.

[NTP] National Toxicology Program (US). 1993. Testing status of agents at NTP: Benzidine, 2,2′-disulfonic acid, CASRN: 117-61-3. Research Triangle Park (NC): US Department of Health and Human Services, National Toxicology Program.

[NTP] National Toxicology Program (US). 1998. Report on carcinogens background document for dyes metabolized to 3,3′-dimethoxybenzidine. Research Triangle Park (NC): US Department of Health and Human Services, National Toxicology Program.

[NTP] National Toxicity Program (US). 2011. Report on carcinogens. 12th ed. Research Triangle Park (NC): US Department of Health and Human Services, National Toxicology Program. p. 1–499.

O’Brien PJ, Gregory B. 1985. Peroxidase catalyzed *N*-oxidation and activation of *N*,*N*′,*N*,*N*′-tetramethylbenzidine. In: J.W. Gorrod, editor. Biological oxidation of nitrogen in organic molecules. London (GB): Ellis Horwood Publishing. p. 337–343.

Oda Y. 2004. Analysis of the involvement of human *N*-acetyltransferase 1 in the genotoxic activation of bladder carcinogenic arylamines using a SOS/umu assay system. Mutat Res 554(1–2):399–406.

Oda Y, Yamazaki H, Watanabe M, Nohmi T, Shimada T. 1995. Development of high sensitive umu test system: rapid detection of genotoxicity of promutagenic aromatic amines by *Salmonella typhimurium* strain NM2009 possessing high *O*-acetyltransferase activity. Mutat Res 334(2):145–156.

[OECD] Organisation for Economic Co-operation and Development. 1997. OECD guidelines for testing of chemicals: Test No. 471—Bacterial reverse mutation test [Internet]. Paris (FR): OECD. [cited 2012 Feb 9]. Available from: www.oecd.org/dataoecd/18/31/1948418.pdf

[OECD] Organisation for Economic Co-operation and Development. 2004. Emission scenario document on textile finishing industry [Internet]. Paris (FR): OECD, Environment Directorate. (Series on Emission Scenario Documents No. 7). Report No.: ENV/JM/MONO(2004)12. Available from: http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=ENV/JM/MONO(2004)12&doclanguage=en

OECD QSAR Toolbox [Read-across tool]. 2011. Version 2.3. Paris (FR): Organisation for Economic Co-operation and Development, Environment Directorate. [cited 2012]. Available from: www.oecd.org/document/23/0,3343,en_2649_34379_33957015_1_1_1_1,00.html

Ogugbue CJ, Oranusi NA. 2006. Toxicity of azo dyes to the freshwater shrimp (*Desmocaris trispinosa*). Int J Nat Appl Sci 1(1):37–44.

Okajima E, Hiranatsu K, Ighu T, Matsujima S, Yamada K, Ari M. 1975. Multiple tumours in rats after oral administration of the benzidine type dye, direct deep black EX. Igaku No Ayumi 92:291–292. [cited in IARC 1982a].

Øllgaard H, Frost L, Galster J, Hansen OC. 1998. Survey of azo-colorants in Denmark: consumption, use, health and environmental aspects. (Miljøprojekt No. 509). Copenhagen (DK): Danish Ministry of Environment and Energy, Environmental Protection Agency. Available from: www2.mst.dk/udgiv/publications/1999/87-7909-548-8/pdf/87-7909-546-1.pdf

Omar AE. 1983. Evaluation of the mutagenic potential of some compounds using *Salmonella typhimurium*. Environ Int 9(1):51–54.

Oster G. 1955. Dye binding to high polymers. J Polym Sci 16:235–244.

Pagga U, Brown D. 1986. The degradation of dyestuffs: Part II. Behavior of dyestuffs in aerobic biodegradation tests. Chemosphere 15(4):479–491.

Papacharalampous NX. 1957. Befunde an der Ratte nach langfristigen Versuchen mit intraperitonealen Injektionen von Trypanblau. Beitr Path Anat 117:85–89. [cited in IARC 1975].

Papacharalampous NX. 1966. Die Ubertragung von Reticulosarkomen der Ratte nach Trypanblauinjektionen. Frankfurt Z Path 75:74–77. [cited in IARC 1975].

Perrin DD, compiler. 1965. Dissociation constants of organic bases in aqueous solution. London (GB): Butterworths.

[PhysProp] Interactive PhysProp Database [database on the Internet]. 2006. Syracuse (NY): Syracuse Research Corporation. [cited 2012 Aug 27]. Available from: www.syrres.com/what-we-do/databaseforms.aspx?id=386

Platzek T, Lang C, Grohmann G, Gi US, Baltes W. 1999. Formation of a carcinogenic aromatic amine from an azo dye by human skin bacteria *in vitro*. Hum Exp Toxicol 18(9):552–559.

Pliss GB. 1963. On some regular relationships between carcinogenicity of aminodiphenyl derivatives and the structure of substance. Acta Unio Int Contra Cancrum 19:499–501. [cited in IARC 1974a, 2010b].

Pliss GB. 1965. On the carcinogenic properties of *o*-toluidine and dianisidine. Gig Tr Prof Zabol 9:18–22. [cited in IARC 1974a, 2010b].

Pliss GB, Zabezhinsky MA. 1970. Carcinogenic properties of orthotolidine (3,3′-dimethylbenzidine). J Natl Cancer Inst 45(2):283–295. [cited in IARC 2010d].

Prival MJ, Mitchell VD. 1982. Analysis of a method for testing azo dyes for mutagenic activity in *Salmonella typhimurium* in the presence of flavin mononucleotide and hamster liver S9. Mutat Res 97(2):103–116.

Prival MJ, Bell SJ, Mitchell VD, Peiperl MD, Vaughan VL. 1984. Mutagenicity of benzidine and benzidine-congener dyes and selected monoazo dyes in a modified *Salmonella* assay. Mutat Res 136(1):33–47.

Probst GS, McMahon RE, Hill LE, Thompson CZ, Epp JK, Neal SB. 1981. Chemically-induced unscheduled DNA synthesis in primary rat hepatocyte cultures: a comparison with bacterial mutagenicity using 218 compounds. Environ Mutagen 3(1):11–32.

Rajaguru P, Fairbairn LJ, Ashby J, Willington MA, Turner S, Woolford LA, Chinnasamy N, Rafferty JA. 1999. Genotoxicity studies on the azo dye Direct Red 2 using the *in vivo* mouse bone marrow micronucleus test. Mutat Res 444(1):175–180.

RAPEX. 2012. RAPEX—Latest notifications [Internet]. Brussels (BE): European Commission, Directorate-General Health & Consumers. [cited 2012 Jul 12]. Available from: http://ec.europa.eu/consumers/dyna/rapex/rapex_archives_en.cfm

Razo-Flores E, Luijten M, Donlon B, Lettinga G, Field J. 1997. Biodegradation of selected azo dyes under methanogenic conditions. Water Sci Technol 36(6–7):65–72.

Reid TM, Morton KC, Wang CY, King CM. 1983. Conversion of congo red and 2-azoxyfluorene to mutagens following *in vitro* reduction by whole-cell rat cecal bacteria. Mutat Res 117(1–2):105–112.

Reid TM, Morton KC, Wang CY, King CM. 1984. Mutagenicity of azo dyes following metabolism by different reductive/oxidative systems. Environ Mutagen 6(5):705–717.

Ricca Chemical Co. 2008. Material Safety Data Sheet: Metanil Yellow [Internet]. Arlington (TX): MSDSonline. [cited 2012 Aug]. Available from: www.msdsonline.com [restricted access]

Rinde E. 1974. Measurement of free aromatic amines in urine and application to the metabolism of 4 benzidine derived azo dyes [Master's thesis]. Morristown (NJ): Allied Chemical Corporation. Case No.: 84003A; Microfiche No.: 215154.

Rinde E, Troll W. 1975. Metabolic reduction of benzidine azo dyes to benzidine in the rhesus monkey. J Natl Cancer Inst 55(1):181–182.

Robens JF, Dill GS, Ward JM. 1980. Thirteen-week subchronic toxicity studies of Direct Blue 6, Direct Black 38, and Direct Brown 95 dyes. Toxicol Appl Pharmacol 54(3):431–442.

Robertson JA, Harris WJ, McGregor DB. 1982. Mutagenicity of azo dyes in the *Salmonella*/activation test. Carcinogenesis 3(1):21–25.

Rofe P. 1957. Azo dyes and Heinz bodies. Br J Ind Med 14:275–280.

Rosner E. 1999a. 14-day oral toxicity (gavage) study in the rat. Itingen (CH): RCC Ltd. RCC Report No.: 736288.

Rosner E. 1999b. 14-day oral toxicity (gavage) study in the rat. Itingen (CH): RCC Ltd. RCC Report No.: 746460.

Rudd CJ, Mitchell AD, Spalding J. 1983. L5178Y mouse lymphoma cell mutagenesis assay of coded chemicals incorporating analyses of the colony size distributions. Environ Mutagen 5:419 (Abstract No. Cd-19).

Saffiotti U, Cefis F, Montesano R, Sellakunar A. 1967. Induction of bladder cancer in hamsters fed aromatic amines. In: Bladder cancer: a symposium. Birmingham (AL): Aesculapius Publishing Co. p. 129–135. [cited in NTP 1998; IARC 2010b, d].

Sagelsdorff P, Haenggi R, Heuberger B, Joppich-Kuhn R, Jung R, Weideli HJ, Joppich M. 1996. Lack of bioavailability of dichlorobenzidine from diarylide azo pigments: molecular dosimetry for hemoglobin and DNA adducts. Carcinogenesis 17(3):507–114.

Sasaki YF, Fujikawa K, Ishida K, Kawamura N, Nishikawa Y, Ohta S, Satoh M, Madarame H, Ueno S, Susa N, Matsusaka N, Tsuda S. 1999. The alkaline single cell gel electrophoresis assay with mouse multiple organs: results with 30 aromatic amines evaluated by the IARC and U.S. NTP. Mutat Res 440(1):1–18.

[SCCNFP] Scientific Committee on Cosmetic Products and Non-food Products. 2002. SCCNFP/0495/01 Opinion concerning the safety review of the use of certain azo-dyes in cosmetic products, adopted by the SCCNFP during the 19th plenary meeting of 27 February 2002.

Schieferstein GJ, Shinohara Y, Allen RR, Sheldon W, Greenman DL, Allaben WT. 1989. Carcinogenicity study of 3,3′-dimethylbenzidine dihydrochloride in BALB/c mice. Food Chem Toxicol 27(12):801–806.

Schieferstein GJ, Sheldon WG, Allen RR, Greenman DL, Allaben WT. 1990. Oncogenic evaluation of 3,3′-dimethoxybenzidine dihydrochloride in BALB/c mice. J Am Coll Toxicol 9(1):71–77.

Sellakumar AR, Montesano R, Saffiotti U. 1969. Aromatic amines carcinogenicity in hamsters. Proc Am Assoc Cancer Res 10:78. [cited in IARC 1974a; NTP 1998].

Sigma-Aldrich. 2010. Material Safety Data Sheet: [Internet]. St. Louis (MO): Sigma-Aldrich. Available from: www.sigmaaldrich.com/safety-center.html

Sigma-Aldrich. 2012a. Material Safety Data Sheet: Nitrotetrazolium Blue chloride [Internet]. St. Louis (MO): Sigma-Aldrich. Available from: www.sigmaaldrich.com/safety-center.html

Sigma-Aldrich. 2012b. Material Safety Data Sheet: *o*-Tolidine [Internet]. St. Louis (MO): Sigma-Aldrich. Available from: www.msdsonline.com [restricted access]

Sigma-Aldrich. 2012d. Material Safety Data Sheet: *o*-Tolidine dihydrochloride [Internet]. St. Louis (MO): Sigma-Aldrich. Available from: www.sigmaaldrich.com/safety-center.html

Sigma-Aldrich. 2012d. Material Safety Data Sheet: 3,3'-Dimethyl-4'-Biphenylene Diisocyanate [Internet]. St. Louis (MO): Sigma-Aldrich. Available from: www.sigmaaldrich.com/safety-center.html

Simpson CL. 1952. Trypan blue–induced tumours of rats. Br J Exp Pathol 33:524–528.

Singh GB, Khanna SK. 1979. Toxicity studies in rats fed orange II. Indian J Exp Biol 17(10):1100–1102.

Singh RL, Khanna SK, Singh GB. 1987. Acute and short-term toxicity studies in orange II. Vet Hum Toxicol 29(4):300–304.

Smith JH, Bomberger DC Jr, Haynes DL. 1980. Prediction of the volatilization rates of high-volatility chemicals from natural water bodies. Environ Sci Technol 14:1332–1337 [cited in Canada 1993a].

SMS Technology Co., Ltd. 2012. Safety Data Sheet for Navacid Acid Scarlet FGW. [Internet]. Taipei City, Taiwan Available from: http://dns.intonline.org/product/navacid_3.htm.

Smyth, Shirley Anne. 2012. WASTEWATER 101: for CMP Monitoring, Presentation to SRAD Gatineau QC, SRAD/EPD, 16 Oct 2012.

[SPIN] Substances in Preparations in Nordic Countries [database on the Internet]. 20010 Copenhagen (DK): Nordic Council of Ministers. [cited 2012 Aug]. Available from: http://90.184.2.100/DotNetNuke/

Spitz S, Maguigan WH, Dobriner K. 1950. The carcinogenic action of benzidine. Cancer 3(5):789–804. [cited in IARC 2010d].

[SRC] Datalog Chemical Search [online database]. 2011. Syracuse (NY): SRC Inc. [cited 2012 Aug]. Available from: http://srcinc.com/what-we-do/databaseforms.aspx?id=379.

[STP] STP Model [sewage treatment plant removal model]. 2006. Version 2.11. Peterborough (ON): Trent University, Canadian Centre for Environmental Modelling and Chemistry. Available from: www.trentu.ca/academic/aminss/envmodel.

Study Submission. 2007. Unpublished confidential studies submitted to Environment Canada under the Chemicals Management Plan initiative. Gatineau (QC): Environment Canada, Program Development and Engagement Division

Takagi Y, Endoh O, Goto S, Kohzaka K, Matsushita H. 1995. Mutagenicity of diaminodiphenylmethane and related compounds. J Environ Chem 5(4):841–845.

Tanaka K, Marui S, Mii T. 1980. Mutagenicity of extracts of urine from rats treated with aromatic amines. Mutat Res 79(2):173–176.

Tanaka K, Mii T, Marui S. 1982. Some aspects of metabolism and mutagenicity of *o*-tolidine and an *o*-tolidine-based azo dye. Ind Health 20(3):227–235.

Therapeutic Guidelines Ltd. 2008. Lund and Browder chart for calculating the percentage of total body surface area burnt (Fig 14.19) [Internet]. West Melbourne (AU): Therapeutic Guidelines Ltd. Available from: www.tg.org.au/etg_demo/etg-lund-and-browder.pdf

Tonogai Y, Ogawa S, Ito Y, Iwaida M. 1982. Actual survey on TL_m (median tolerance limit) values of environmental pollutants, especially on amines, nitriles, aromatic nitrogen compounds and artificial dyes. J Toxicol Sci 7:193–203.

Tsuda T, Kojima M, Harada H, Nakajima A, Aoki S. 1997. Acute toxicity, accumulation and excretion of organophosphorous insecticides and their oxidation products in killifish. Chemosphere 35(5):939–949.

Tsuda S, Matsusaka N, Madarame H, Ueno S, Susa N, Ishida K, Kawamura N, Sekihashi K, Sasaki YF. 2000. The comet assay in eight mouse organs: results with 24 azo compounds. Mutat Res 465(1–2):11–26.

Tsuji S, Tonogai Y, Ito Y, Kanoh S. 1986. The influence of rearing temperatures on the toxicity of various environmental pollutants for killifish (*Oryzias latipes*). J Hyg Chem 32:46–53.

Ulland BM, Maronpot RR, Lemen JK, Mennear JH. 1989. Transplantation studies of preputial gland and epithelial skin neoplasms derived from benzidine-based dye carcinogenicity assays in Fischer 344 male rats. Toxicol Pathol 17(1 Pt 1):50–56.

[Ullmann's Encyclopedia] Ullmann's encyclopedia of industrial chemistry [online]. 2010. John Wiley & Sons, Inc. Available from: http://onlinelibrary.wiley.com/book/10.1002/14356007

[US EPA] US Environmental Protection Agency. 1987a. Health and environmental effects profile for Direct Black 38. Washington (DC): US EPA. Report No.: EPA/600/x-87/116.

[US EPA] US Environmental Protection Agency. 1987b. Health and environmental effects profile for Direct Brown 95. Washington (DC): US EPA. Report No.: EPA/600/x-87/117.

USEPA (1994), “Manufacture and use of printing ink,” U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Chemical Engineering Branch, Washington, DC., September 22, 1994.

[US EPA] US Environmental Protection Agency. 1996. Best management practices for pollution prevention in the textile industry. Cincinnati (OH): US Environmental Protection Agency, Office of Research and Development. Report No.: EPA/625/R-96/004.

[US EPA] US Environmental Protection Agency. 2010 TSCA [*Toxic Substances Control Act*] New Chemicals Program (NCP) chemical categories. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics. p. 2–4. Available from: www.epa.gov/oppt/newchems/pubs/chemcat.htm

[US EPA] US Environmental Protection Agency. 2005. Inventory Update Reporting program. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics. Available from: http://www.epa.gov/cdr/tools/previouslycollected.html

[US EPA] US Environmental Protection Agency. 2010. Dyes derived from benzidine and its congeners [Internet]. Available from: www.epa.gov/oppt/existingchemicals/pubs/actionplans/DCB%20Action%20Plan_06232010.noheader.pdf

[US EPA] US Environmental Protection Agency. 2012a. Standard operating procedures for residential pesticide exposure assessment. Available from: www.epa.gov/pesticides/science/EPA-OPP-HED_Residential%20SOPS_Feb2012.pdf

[US EPA] US Environmental Protection Agency. 2012b. Benzidine-based chemical substances; di-*n* [Internet]. A proposed rule by the Environmental Protection Agency on 03/28/2012. 40 CFR 721. Fed Regist 77(6):18752–18766. Available from: www.federalregister.gov/articles/2012/03/28/2012-7208/benzidine-based-chemical-substances-di-n#p-3

[US EPA] US Environmental Protection Agency. 2013. Benchmark Dose Software (BMDS version 2.3.1), Environmental Protection Agency. Available from: http://www.epa.gov/ncea/bmds/.

Venitt S, Crofton-Sleigh C. 1979. Bacterial mutagenicity tests of phenazine methosulfate and 3 tetrazolium salts. Mutat Res 68(2):107–116.

Venturini S, Tamaro M. 1979. Mutagenicity of anthraquinone and azo dyes in Ames' *Salmonella typhimurium* test. Mutat Res 68(4):307–312.

Von der Hude W, Behm C, Gürtler R, Basler A. 1988. Evaluation of the SOS chromotest. Mutat Res 203(2):81–94.

Waalkens DH, Joosten HFP, Yih TD, Hoekstra A. 1981. Mutagenicity studies with *o*-tolidine and 4,4′-tetramethyldiaminodiphenylmethane. Mutat Res 89(3):197–202.

[WATERNT] Water Solubility Program [Estimation Model]. 2010. Version 1.01. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

Weber EJ. 1991. Studies of benzidine-based dyes in sediment–water systems. Environ Toxicol Chem 10:609–618.

Williams JH. 1999. Regulations on additions of sludge-borne metals to soil and their adaptation to local conditions. In: L'Hermite P, editor. Treatment and use of sewage sludge and liquid agricultural wastes. London (GB): Elsevier Applied Science, p. 243–250.

Woodruff RC, Mason JM, Valencia R, Zimmering S. 1985. Chemical mutagenesis testing in *Drosophila*. V. Results of 53 coded compounds tested for the National Toxicology Program. Environ Mutagen 7:677–702.

Woolrich PF. 1973. Monoisocyanates, diisocyanates and polyisocyanates: engineering, medical control and toxicologic considerations. Kalamazoo (MI): The Upjohn Co. p. 21. Technical Bulletin No.: 106.

[WSKOWWIN] Water Solubility for Organic Compounds Program for Microsoft Windows [Estimation Model]. 2010. Version 1.42. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

Yen CC, Perenich TA, Baughman GL. 1991. Fate of commercial disperse dyes in sediments. Environ Toxicol Chem 10:1009–1017.

Yi J, Chen Y, Wang L. 1993. Phosphorus-32 postlabeling test of DNA adducts formed in the livers of rats treated with carcinogen. J Xi'an Med Univ 14(1):17–20.

Yoon JS, Mason JM, Valencia R, Woodruff RC, Zimmering S. 1985. Chemical mutagenesis testing in *Drosophila*. IV. Results of 45 coded compounds tested for the National Toxicology Program. Environ Mutagen 7(3):349–367.

Yoshikawa K, Nohmi T, Harada R, Ishidate MJR, Inokawa Y. 1979. Differential mutagenicities of triamino benzenes against *Salmonella typhimurium* TA98 in the presence of S9 fractions from polychlorinated biphenyls, phenobarbital or 3-methylcholanthrene-pretreated rats, hamsters and mice. J Toxicol Sci 4(4):317–326.

You Z, Brezzell MD, Das SK, Espadas-Torre MC, Hooberman BH, Sinsheimer JE. 1993. Ortho-substituent effects on the *in vitro* and *in vivo* genotoxicity of benzidine derivatives. Mutat Res 319(1):19–30.

Zeiger E, Anderson B, Haworth S. 1987. *Salmonella* mutagenicity tests: III. Results from the testing of 255 chemicals. Environ Mutagen 9(Suppl 9):1–110.

Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. 1988. *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. Environ Mol Mutagen 11(Suppl 12):1–157.

Zeilmaker MJ, Kroese ED, van Haperen P, van Veen MP, Bremmer HJ, van Kranen HJ, Wouters MFA, Janus JA. 1999. Cancer risk assessment of azo dyes and aromatic amines from garment and footwear. Bilthoven (NL): Rijkinstituut voor Volkgezondheid en Milieu (National Institute for Public Health and the Environment). RIVM Report No.: 601503 014. Available from: www.rivm.nl/bibliotheek/rapporten/601503014.html

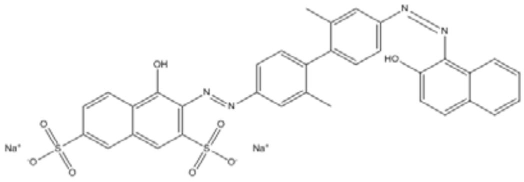
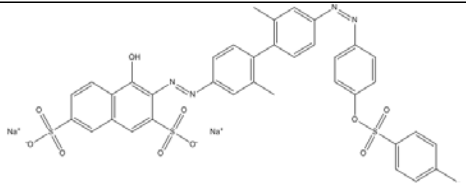
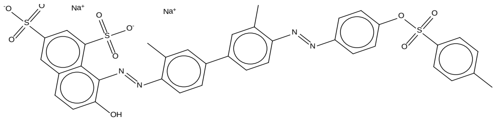
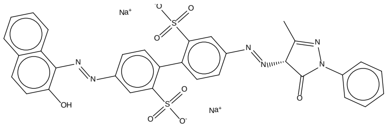
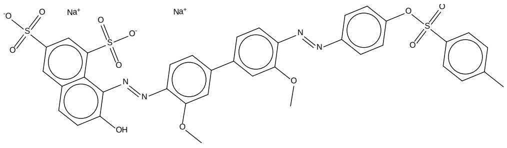
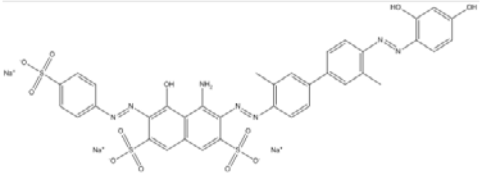
Zhou Y, You X, Ye X. 1987. Mutagenicity of benzidines and their congener derivative dyes. Huanjing Kexue 8(2):31–34. [cited in CCRIS 2012].

Zimmering S, Mason JM, Valencia R. 1989. Chemical mutagenesis testing in *Drosophila*. VII. Results of 22 coded compounds tested in larval feeding experiments. Environ Mol Mutagen 14:245–251.

Appendices

Appendix A. Supplementary Data Tables

Table A-1. Substance identities for individual Benzidine-based Acid Dyes

CAS RN	Chemical structure	Chemical formula (molecular weight in g/mol)
3701-40-4		$C_{34}H_{24}N_4O_8S_2Na_2$ (726.69)
6358-57-2		$C_{37}H_{30}N_4O_{10}S_3$, 2Na (830.82)
6459-94-5		$C_{37}H_{28}N_4O_{10}S_3$, 2Na (830.82)
6470-20-8		$C_{32}H_{22}N_6O_8S_2Na_2$ (728.67)
6548-30-7		$C_{37}H_{28}N_4O_{12}S_3Na_2$ (862.81)
68318-35-4		$C_{36}H_{26}N_7O_{12}S_3Na_3$ (913.80)

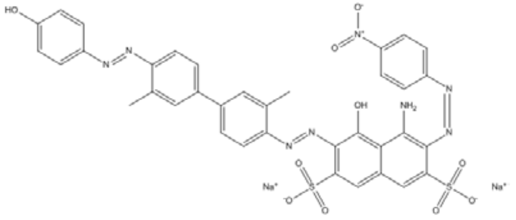
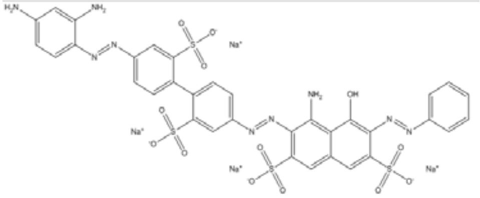
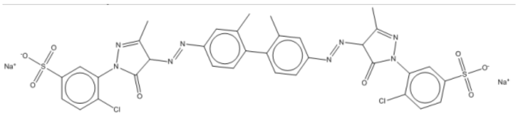
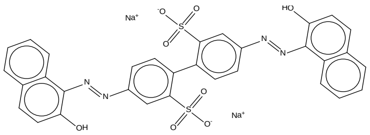
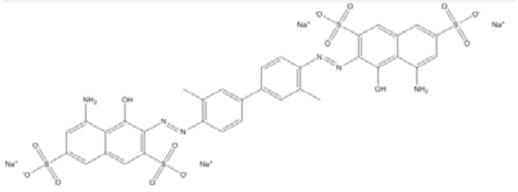
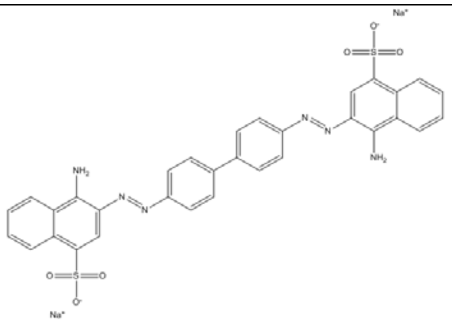
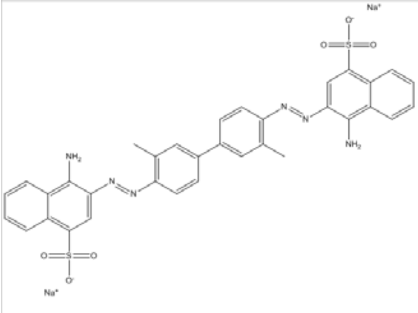
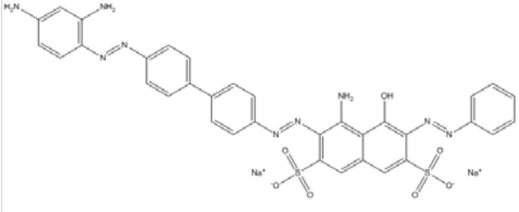
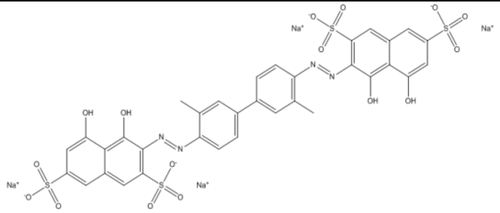
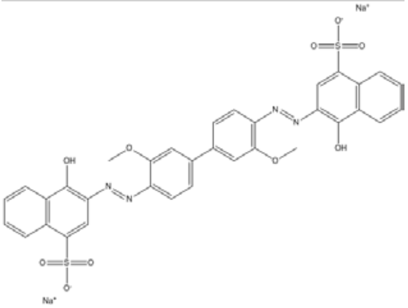
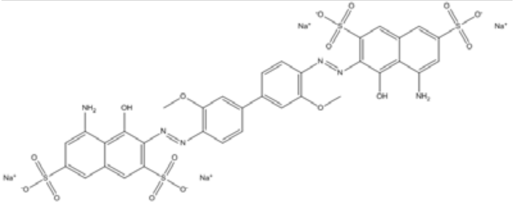
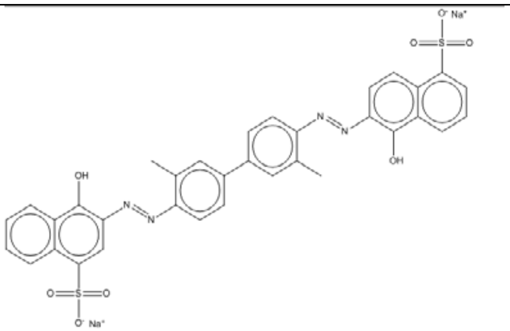
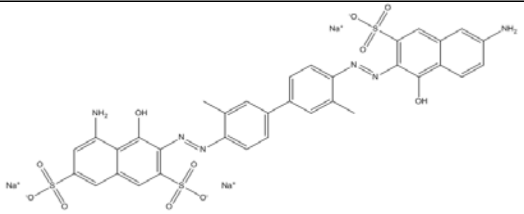
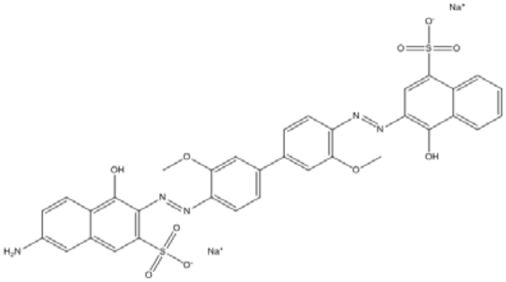
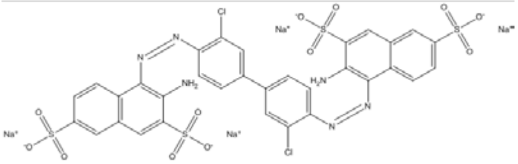
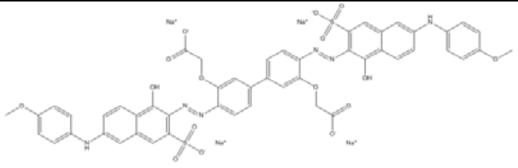
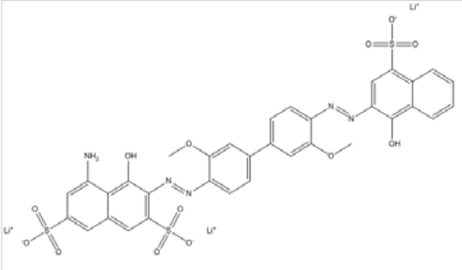
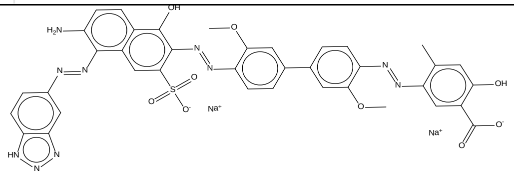
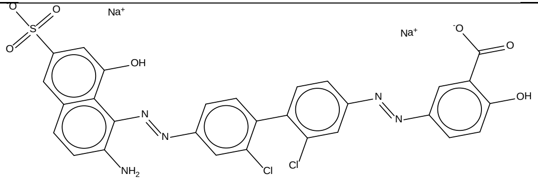
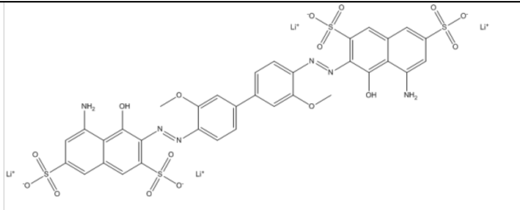
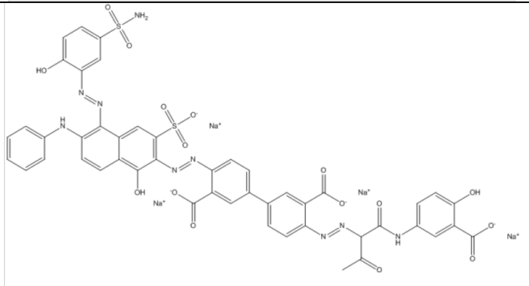
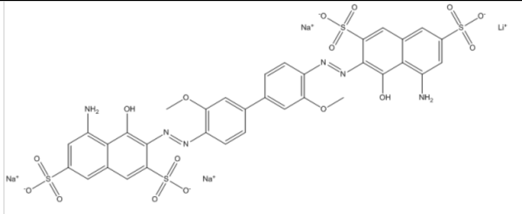
CAS RN	Chemical structure	Chemical formula (molecular weight in g/mol)
68400-36-2		$C_{36}H_{26}N_8O_{10}S_2Na_2$ (840.75)
83221-63-0		$C_{34}H_{26}N_9O_{13}S_4Na$ (919.87)
89923-60-4		$C_{34}H_{26}Cl_2N_8O_8S_2Na_2$ (855.64)
10169-02-5		$C_{32}H_{20}N_4O_8S_2Na_2$ (698.64)

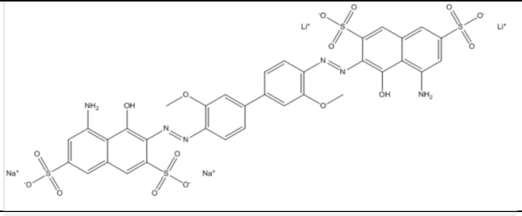
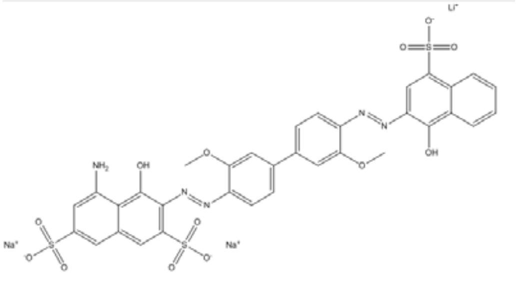
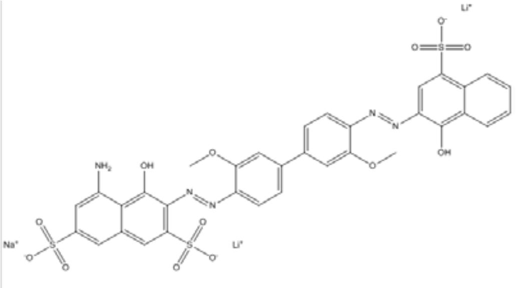
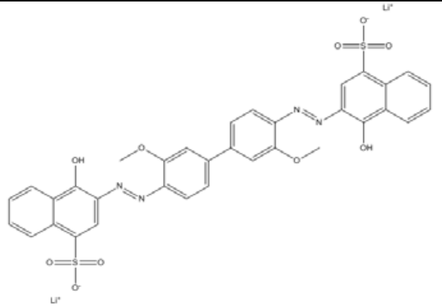
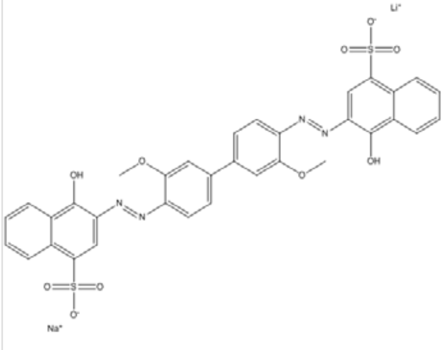
Table A-2. Substance identities for individual Benzidine-based Direct Dyes

CAS RN	Chemical structure	Chemical formula (molecular weight in g/mol)
72-57-1		$C_{34}H_{24}N_6O_{14}S_4Na_4$ (960.80)

CAS RN	Chemical structure	Chemical formula (molecular weight in g/mol)
573-58-0		$C_{32}H_{22}N_6O_6S_2Na_2$ (696.67)
992-59-6		$C_{34}H_{26}N_6O_6S_2Na_2$ (724.72)
1937-37-7		$C_{34}H_{27}N_9O_7S_2$ (737.77)
2150-54-1		$C_{34}H_{22}N_4O_8S_2Na_4$ (770.65)
2429-71-2		$C_{34}H_{24}N_4O_9S_2Na_2$ (742.69)

CAS RN	Chemical structure	Chemical formula (molecular weight in g/mol)
2429-74-5		$C_{34}H_{28}N_5O_{10}S_2Na_4$ (922.75)
6420-06-0		$C_{34}H_{24}N_4O_8S_2Na_2$ (726.69)
6420-22-0		$C_{34}H_{25}N_6O_{11}S_3Na_3$ (858.76)
6449-35-0		$C_{34}H_{25}N_5O_{10}S_2Na_2$ (773.70)
6548-29-4		$C_{32}H_{20}CL_2N_6O_6S_2Na_2$ (765.56)
6655-95-4		$C_{50}H_{36}N_6O_{16}S_2Na_4$ (1132.95)

CAS RN	Chemical structure	Chemical formula (molecular weight in g/mol)
67923-89-1		$C_{34}H_{24}N_5O_{13}S_3Li_3$ (827.60)
70210-28-5		$C_{38}H_{28}N_{10}O_9SNa_2$ (846.75)
71215-83-3		$C_{29}H_{17}Cl_2N_5O_7SNa_2$ (696.43)
71550-22-6		$C_{34}H_{24}N_6O_{16}S_4Li_4$ (928.60)
72252-59-6		$C_{47}H_{31}N_9O_{16}S_2Na_4$ (1133.90)
75659-72-2		$C_{34}H_{24}N_6O_{16}S_4Na_3Li$ (976.75)

CAS RN	Chemical structure	Chemical formula (molecular weight in g/mol)
75659-73-3		$C_{34}H_{24}N_6O_{16}S_4Na_2Li_2$ (960.70)
75673-18-6		$C_{34}H_{25}N_5O_{13}S_3Na_2$ (860.76)
75673-19-7		$C_{34}H_{26}N_5O_{13}S_3Na$ (831.78)
75673-34-6		$C_{34}H_{24}N_4O_{10}S_2Li_2$ (726.59)
75673-35-7		$C_{34}H_{24}N_4O_{10}S_2NaLi$ (742.64)

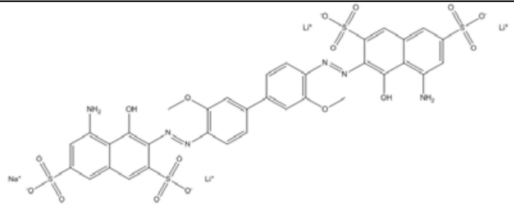
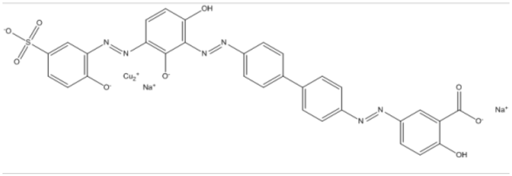
CAS RN	Chemical structure	Chemical formula (molecular weight in g/mol)
75752-17-9		$C_{34}H_{24}N_6O_{16}S_4NaLi_3$ (944.65)
16071-86-6		$C_{31}H_{18}N_6O_9SNa_2Cu$ (760.11)

Table A-3. Substance identities for the Benzidine-based Cationic Indicators

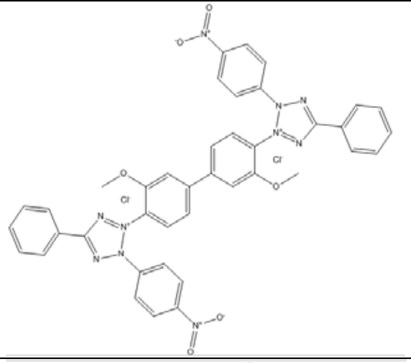
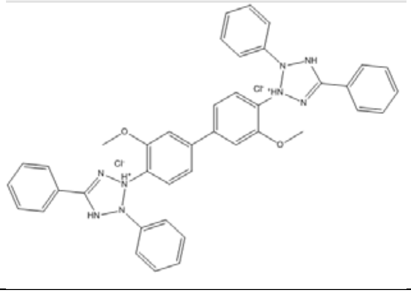
CAS RN	Chemical structure	Chemical formula (molecular weight in g/mol)
298-83-9		$C_{40}H_{30}Cl_2N_{10}O_6$ (817.65)
1871-22-3		$C_{40}H_{30}N_8O_2Cl_2$ (654.74)

Table A-4. Experimental physical and chemical properties for individual Benzidine-based Acid Dyes (with data) including substances used for read-across

CAS RN	Property	Value	Reference
Acid Red 111	Physical state	Red Powder (formulation of Lanasyn Scarlet F-3GL 103)	Study Submission 2007
Acid Red 111	Melting point (°C)	170–190 (formulation of Lanasyn Scarlet F-3GL 103)	Study Submission 2007
Acid Red 111	Density (kg/m^3)	390	Study Submission 2007
Acid Red 111	Water solubility (mg/L)	65 000	SMS Technology Co., Ltd. 2012
Acid Red 111	Water solubility (mg/L)	25 000 (at 80°C)	Study Submission 2007
Acid Red 114	Melting point (°C)	185	MITI 1992
Acid Red 114	Water solubility (mg/L)	> 500	MITI 1992
Acid Yellow 23 (read-across for log K_{ow})	Melting point (°C)	> 300	Acros Organics 2006
Acid Yellow 23 (read-across for log K_{ow})	Water solubility (mg/L)	200 000	Marmion 1991
Acid Yellow 23 (read-across for log K_{ow})	Water solubility (mg/L)	300 000	Green 1990
Acid Yellow 23 (read-across for log K_{ow})	Water solubility (mg/L)	> 2%	MITI 1992
Acid Yellow 23 (read-across for log K_{ow})	Log K_{ow}	−0.017	CITI 1992
Acid Yellow 36 (read-across for log K_{ow})	Water solubility	Soluble	Ricca Chemical Co. 2008; Acros Organics 2009a
Acid Yellow 36 (read-across for log K_{ow})	Log K_{ow}	0.7	Tonogai et al. 1982
Acid Orange	Melting point (°C)	164	Acros Organics 2009b

CAS RN	Property	Value	Reference
7 (read-across for log K_{ow})			
Acid Orange 7 (read-across for log K_{ow})	Log K_{ow}	0.57	Tonogai et al. 1982
Acid Orange 7 (read-across for log K_{ow})	Water solubility (mg/L)	116 000	Acros Organics 2009b
Acid Orange 7 (read-across for log K_{ow})	Water solubility (mg/L)	50 000	Merck Index 1989

Table A-5. Experimental physical and chemical properties for individual Benzidine-based Direct Dyes (with data)

CAS RN	Property	Value	Reference
Direct Blue 14	Physical state	Bluish-grey powdered solid	ChemicalBook 2008a
Direct Blue 14	Melting point (°C)	> 300 (decomposes)	ChemicalBook 2008a
Direct Blue 14	Melting point (°C)	> 300 (decomposes)	CHRIP ©2002-2012
Direct Blue 14	Melting point (°C)	300	Øllgaard et al. 1998
Direct Blue 14	Water solubility (mg/L)	20 000	CHRIP ©2002-2012
Direct Blue 14	Water solubility (mg/L)	10 000	ChemicalBook 2008a
Direct Black 38	Melting point (°C)	109–110	ChemicalBook 2008b
Direct Black 38	Water solubility (mg/L)	93 000	Isik and Sponza 2004
Direct Red 28	Physical state	Brown-red powder	ChemicalBook 2008c
Direct Red 28	Melting point (°C)	> 360	ChemicalBook 2008c; Alfa Aesar ©2011
Direct Red 28	Density (kg/m^3)	995	ChemicalBook 2008c
Direct Red 28	Log K_{ow}	0.77	Tonogai et al. 1982
Direct Red 28	Water solubility (mg/L)	116 000	Dehn 1917
Direct Brown 95	Physical state	Dark brown microcrystals or charcoal black powder	ChemicalBook 2008d
Direct Blue 15	Physical state	Deep purple to dark blue microcrystalline powder	ChemicalBook 2008e
Direct Blue 15	Water solubility (mg/L)	30 000	Brown 1992
Direct Red 2	Melting point (°C)	~290 (decomposes)	Chemexper 2012
Direct Blue 8	Physical state	Bluish-black powder	ChemicalBook 2008f
Direct Violet 28	Physical state	Bluish-black powder	ChemicalBook 2008g
Direct Blue 151	Physical state	Bluish-black powder	ChemicalBook 2008h

Table A-6. Physical and chemical properties for the Cationic Indicators subgroup

CAS RN	Property	Value	Reference
TDBD	Physical state	Yellow crystalline solid	ChemicalBook 2008i
TDBD	Melting point (°C)	255	ChemicalBook 2008i
TDBD	Melting point (°C)	~190	Alfa Aesar ©2011
TDBD	Water solubility (mg/L)	9000	Green 1990
TDBPD	Physical state	Yellow crystals	ChemicalBook 2008j
TDBPD	Melting point (°C)	189	Sigma-Aldrich 2012a
TDBPD	Melting point (°C)	200	Chemical Book 2008j
TDBPD	Water solubility (mg/L)	10 000	Green 1990
Basic Dyes	Log K_{ow}	Low	Øllgaard et al. 1998

Table A-7. Estimated physical and chemical properties for the Benzidine-based Precursors subgroup

CAS RN	Property	Value	Reference
Naphthol AS-BR	Melting point (°C)	246	MPBPWIN 2010
Naphthol AS-BR	Melting point (°C)	350	MPBPWIN 2010
Naphthol AS-BR	Boiling point (ºC)	927.49	MPBPWIN 2010
Naphthol AS-BR	Vapour pressure (Pa)	7.7×10^{-25}	MPBPWIN 2010
Naphthol AS-BR	Henry's Law constant (Pa·m^3/mol)	1.96×10^{-15}	HENRYWIN 2011
Naphthol AS-BR	Log K_{ow}	7.75	KOWWIN 2010
Naphthol AS-BR	Log K_{oc}	1.43×10^5 (MCI method)	KOCWIN 2010
Naphthol AS-BR	Log K_{oc}	8.27×10^5 (K_{ow} method)	
Naphthol AS-BR	Log K_{oa}	25.853	KOAWIN 2010
Naphthol AS-BR	Water solubility (mg/L)	8.97×10^{-6}	WSKOWWIN 2010
Naphthol AS-BR	Water solubility (mg/L)	1.44×10^{-5}	WATERNT 2010
TCDB	Melting point (°C)	250.21	MPBPWIN 2010
TCDB	Boiling point (ºC)	580.51	MPBPWIN 2010
TCDB	Vapour pressure (Pa)	1.12×10^{-10}	MPBPWIN 2010
TCDB	Henry's Law constant (Pa·m^3/mol)	5.81×10^{-9}	HENRYWIN 2011
TCDB	Log K_{ow}	5.13	KOWWIN 2010
TCDB	Log K_{oc}	2.2 (MCI method)	KOCWIN 2010
TCDB	Log K_{oc}	5.47 (K_{ow} method)	

TCDB	Log K_{oa}	16.760	KOAWIN 2010
TCDB	Water solubility (mg/L)	0.2588	WSKOWWIN 2010
TCDB	Water solubility (mg/L)	32.801	WATERNT 2010

Abbreviation: MCI, molecular connectivity index

Table A-8. Physical and chemical properties for the Benzidine Derivatives subgroup

Chemical	Property	Value or range	Reference
3,3′-DMB	Physical state	Light brown powder	Sigma-Aldrich 2012b
3,3′-DMB	Melting point (°C)	128–132	Alfa Aesar ©2011
3,3′-DMB	Melting point (°C)	131.5	PhysProp 2006
3,3′-DMB	Melting point (°C)	129–131	Merck Index 2006
3,3′-DMB	Melting point (°C)	147.85	MPBPWIN 2010
3,3′-DMB	Boiling point (ºC)	200	ACGIH 1986
3,3′-DMB	Boiling point (ºC)	339	PhysProp 2006
3,3′-DMB	Boiling point (ºC)	300	Hawley 1981
3,3′-DMB	Boiling point (ºC)	393.08	MPBPVPWIN 2010
3,3′-DMB	Density (kg/m^3)	1234	ICSC 1998
3,3′-DMB	Vapour pressure (Pa)	9.23×10^{-5} (6.92×10^{-7} mmHg)	Neely and Blau 1985
3,3′-DMB	Vapour pressure (Pa)	2.74×10^{-2} (2.06×10^{-5} mmHg)	MPBPWIN 2010
3,3′-DMB	Henry’s Law constant (Pa·m^3/mol)	6.38×10^{-6} (Bond estimation method) 8.21×10^{-6} (Group contribution method)	HENRYWIN 2011
3,3′-DMB	Henry’s Law constant	6.37×10^{-7} (6.29×10^{-11} atm·m^3/mol)	Meylan and Howard 1991

Chemical	**Property**	**Value or range**	**Reference**
3,3′-DMB	(Pa·m^3/mol)	2.59 × 10^{-2} (2.56 × 10^{-7} atm·m^3/mol) (EVA method)[a]	HENRYWIN 2011
3,3′-DMB	Log K_{ow}	2.34	Hansch et al. 1995
3,3′-DMB	Log K_{ow}	2.39	MITI 1992
3,3′-DMB	Log K_{ow}	3.02	KOWWIN 2010
3,3′-DMB	Log K_{ow}	2.43 (EVA method)[b]	KOWWIN 2010
3,3′-DMB	Log K_{oc}	2.17 (from log K_{ow}) 3.50 (from MCI)	KOCWIN 2010
3,3′-DMB	Log K_{oa}	10.93	KOAWIN 2010
3,3′-DMB	Water solubility (mg/L)	50	MITI 1992
3,3′-DMB	Water solubility (mg/L)	1300	Bowman et al. 1976
3,3′-DMB	Water solubility (mg/L)	27.1	WATERNT 2010
3,3′-DMB	Water solubility (mg/L)	134	MPBPWIN 2010
3,3′-DMB	Water solubility (mg/L)	51.263 (EVA method)[c]	WATERNT 2010
3,3′-DMB	pK_a	4.6	Kawakami et al. 2010
3,3′-DMB	pK_a	pK_{a1} = 4.5 pK_{a2} = 3.4–3.5	Perrin 1965
3,3′-DMB	pK_a	pK_{a1} = 3.3	Kubota and Ezumi 1980
3,3′-DMB·2HCl	Physical state	Light red powder	Sigma Aldrich 2012c
3,3′-DMB·2HCl	Melting point (°C)	340	Sigma Aldrich 2012c
3,3′-DMB·2HCl	Melting point (°C)	210	Beilstein 1984
3,3′-DMB·2HCl	Water solubility (mg/L)	Soluble in water	CHRIP ©2002-2012
3,3′-DMB·2HCl	Water solubility (mg/L)	10 000 – 50 000	ChemBioFinder ©1998–2013
3,3′-DMOB	Physical state	Beige brown crystalline powder	Acros Organics 2007
3,3′-DMOB	Melting point (°C)	137	Lewis 1997
3,3′-DMOB	Melting point (°C)	136–137	Alfa Aesar ©2011
3,3′-DMOB	Melting point (°C)	137–138	Merck Index 2006
3,3′-DMOB	Melting point (°C)	161.6	MPBPWIN 2010

Chemical	**Property**	**Value or range**	**Reference**
3,3′-DMOB	Boiling point (°C)	356	SRC 2011
3,3′-DMOB	Boiling point (°C)	417.2	MPBPWIN 2010
3,3′-DMOB	Vapour pressure (Pa)	9.45×10^{-4} (7.09×10^{-6} mmHg)	MPBPWIN 2010
3,3′-DMOB	Vapour pressure (Pa)	1.66×10^{-5} (1.25×10^{-7} mmHg)	Neely and Blau 1985
3,3′-DMOB	Henry's Law constant ($Pa·m^3/mol$)	1.83×10^{-8} (1.81×10^{-13} $atm·m^3/mol$) (Bond estimation method) 4.72×10^{-6} (4.66×10^{-11} $atm·m^3/mol$) (Group contribution method)	HENRYWIN 2011
3,3′-DMOB	Henry's Law constant ($Pa·m^3/mol$)	4.762×10^{-6} (4.7×10^{-11} $atm·m^3/mol$)	Meylan and Howard 1991
3,3′-DMOB	Henry's Law constant ($Pa·m^3/mol$)	7.45×10^{-5} (7.35×10^{-10} $atm·m^3/mol$) (EVA method)[a]	HENRYWIN 2011
3,3′-DMOB	Log K_{ow}	1.81	Debnath and Hansch 1992
3,3′-DMOB	Log K_{ow}	2.08	KOWWIN 2010
3,3′-DMOB	Log K_{ow}	1.5 (EVA method)[d]	KOWWIN 2010
3,3′-DMOB	Log K_{oc}	1.99 (from log K_{ow}) 2.71 (from MCI)	KOCWIN 2010
3,3′-DMOB	Log K_{oa}	13.211	KOAWIN 2010
3,3′-DMOB	Water solubility (mg/L)	60 mg/L at 25°C	Bowman et al. 1976
3,3′-DMOB	Water solubility (mg/L)	Insoluble	NIOSH 2012
3,3′-DMOB	Water solubility (mg/L)	Slightly soluble	Chemical Book 2008k
3,3′-DMOB	Water solubility (mg/L)	77.54	WATERNT 2010
3,3′-DMOB	Water solubility (mg/L)	146.8 (EVA method)[e]	WATERNT 2010

Chemical	Property	Value or range	Reference
3,3′-DMOB	Water solubility (mg/L)	351	WSKOWWIN 2010
3,3′-DMOB	pK_a	4.7	Kawakami et al. 2010
3,3′-DMOB		4.2 (estimated)	PhysProp 2006
TODI	Physical state	Colourless to pale yellow flakes	Sigma-Aldrich 2012d
TODI	Melting point (°C)	70–72	Chemical Book 2008l
TODI	Melting point (°C)	70	Woolrich 1973
TODI	Melting point (°C)	71	PhysProp 2006
TODI	Melting point (°C)	71.7	ECHA 2012
TODI	Melting point (°C)	115.98	MPBPWIN 2010
TODI	Boiling point (°C)	371–373	ECHA 2012
TODI	Boiling point	314	Kirk-Othmer 1981
TODI	(°C)	364.35	MPBPVPWIN 2010
TODI	Density (kg/m^3)	1330	ECHA 2012
TODI	Density (kg/m^3)	1156 (at 80°C)	Kirk-Othmer 1981
TODI	Vapour pressure (Pa)	2.95×10^{-3} (2.21×10^{-5} mmHg)	MPBPWIN 2010
TODI	Henry's Law constant (Pa·m^3/mol)	NA	NA
TODI	Log K_{ow}	NA	NA
TODI	Log K_{oc}	NA	NA
TODI	Log K_{oa}	10.466	KOAWIN 2010
TODI	Water solubility (mg/L)	NA	NA
TODI	pK_a	Not applicable	Not applicable
4N-TMB	Physical state	Tan-coloured powder	Acros Organics 2008
4N-TMB	Melting point (°C)	193–195	Acros Organics 2008
4N-TMB	Melting point (°C)	193	ChemSpider ©2011

Chemical	**Property**	**Value or range**	**Reference**
4N-TMB	Melting point (°C)	194	SRC 2011
4N-TMB	Melting point (°C)	108.5	MPBPWIN 2010
4N-TMB	Boiling point (°C)	353.7	MPBPWIN 2010
4N-TMB	Vapour pressure (Pa)	2.17×10^{-3} (1.63×10^{-5} mmHg)	Neely and Blau 1985
4N-TMB	Vapour pressure (Pa)	2.41×10^{-4} (1.08×10^{-7} mmHg)	MPBPWIN 2010
4N-TMB	Henry's Law constant ($Pa{\cdot}m^3/mol$)	1.06×10^{-2} (Bond estimation method) (1.05×10^{-7} $atm{\cdot}m^3/mol$)	HENRYWIN 2011
4N-TMB	Henry's Law constant ($Pa{\cdot}m^3/mol$)	4.94×10^{-1} (4.88×10^{-6} $atm{\cdot}m^3/mol$) (EVA method)[a]	HENRYWIN 2011
4N-TMB	Log K_{ow}	4.11	KOWWIN 2010
4N-TMB	Log K_{ow}	3.53 (EVA method)[b]	KOWWIN 2010
4N-TMB	Log K_{oc}	3.17 (from MCI) 3.07 (from log K_{ow}) 2.75 (from corrected log K_{ow})	KOCWIN 2010
4N-TMB	Log K_{oa}	9.48	KOAWIN 2010
4N-TMB	Water solubility (mg/L)	8.23	Meylan et al. 1996
4N-TMB	Water solubility (mg/L)	0.65	WSKOWWIN 2010
4N-TMB	Water solubility (mg/L)	25.85 (EVA method)[d]	WSKOWWIN 2010
4N-TMB	Water solubility (mg/L)	17.87	WATERNT 2010
4N-TMB	Water solubility (mg/L)	33.833 (EVA method)[c]	WATERNT 2010

Abbreviations: EVA, Experimental Value Adjustment; MCI, molecular connectivity index; NA, not available

[a] Estimated with the EVA method using a Henry's Law constant value for benzidine (CAS RN 92-87-5) of 2.2×10^{-2} $Pa{\cdot}m^3/mol$ (Smith et al. 1980).

b Estimated with the EVA method using a log K_{ow} value for benzidine (CAS RN 92-87-5) of 1.34 (Lu et al.1977).
c Estimated with the EVA method using a water solubility value for benzidine (CAS RN 92-87-5) of 500 mg/L (Bowman et al. 1976).
d Estimated with the EVA method using a corrected log K_{ow} value of 3.53.

Table A-9a. Summary of modelled data for degradation of Benzidine-based Acid Dyes[a]

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Atmospheric oxidation (air)	AOPWIN 2010[b]	$t_{½}$ = 0.05–1.38 days	≤ 2
Ozone reaction (air)	AOPWIN 2010[b]	N/A[c]	N/A
Hydrolysis (water)	HYDROWIN 2010[b]	Not in training set	N/A
Primary degradation: Biodegradation (aerobic) (water)	BIOWIN 2008[b] Submodel 4: Expert Survey (qualitative results)	2.15–2.92[d] (biodegrades slowly)	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2008[b] Submodel 3: Expert Survey (qualitative results)	0.48–1.55[d] (biodegrades very slowly)	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2008[b] Submodel 5: MITI linear probability	−2.29 to −1.01[e] (biodegrades very slowly)	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2008[b] Submodel 6: MITI non-linear probability	0[e] (biodegrades very slowly)	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	DS TOPKAT c2005–2009 Probability	N/A	
Ultimate biodegradation: Biodegradation (aerobic) (water)	CATALOGIC ©2004–2011 % BOD	% BOD = 0–20 (biodegrades slowly)	≥ 182

Abbreviations: BOD, biological oxygen demand; MITI, Ministry of International Trade and Industry (Japan); N/A, not applicable

[a] Substances used in this summary include the following CAS RNs: 3701-40-4, 6358-57-2, 6459-94-5, 6548-30-7, 6470-20-8, 68400-36-2, 10169-02-5, 68318-35-4, 83221-63-0 and 89923-60-4.

[b] EPISuite (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.

Table A-9b. Summary of modelled data for degradation of Benzidine-based Direct Dyes[a]

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Atmospheric oxidation (air)	AOPWIN 2010[b]	$t_{½}$ = 0.21–0.71 days	≤ 2
Ozone reaction (air)	AOPWIN 2010[b]	N/A[c]	N/A
Hydrolysis (water)	HYDROWIN 2010[b]	N/A, not in training set	N/A
Primary biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2008[b] Submodel 4: Expert Survey (qualitative results)	2.29–3.2[d] (biodegrades slowly)	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2008[b] Submodel 3: Expert Survey (qualitative results)	0.37–1.37[d] (biodegrades very slowly)	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2008[b] Submodel 5: MITI linear probability	−2.01 to −0.79[e] (biodegrades very slowly)	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2008[b] Submodel 6: MITI non-linear probability	0[e] (biodegrades very slowly)	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	DS TOPKAT c2005–2009 Probability	N/A	
Ultimate biodegradation: Biodegradation (aerobic) (water)	CATALOGIC ©2004–2011 % BOD	% BOD = 0–8 (biodegrades very slowly)	≥ 182

Abbreviations: BOD, biological oxygen demand; MITI, Ministry of International Trade and Industry (Japan); N/A, not applicable;

[a] Substances used in this summary include the following CAS RNs: 72-57-1, 573-58-0, 992-59-6, 2429-71-2, 2429-74-5, 1937-37-7, 2150-54-1, 6420-06-0, 6420-22-0, 6449-35-0, 6548-29-4, 6655-95-4, 67923-89-1, 70210-28-5, 71215-83-3, 72252-59-6, 75659-72-2, 75659-73-3, 75673-18-6, 75673-19-7, 75673-34-6 and 75673-35-7, 75752-17-9, 16071-86-6, and 711550-22-6,

[b] EPISuite (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.

Table A-9c. Summary of modelled data for degradation of Benzidine-based Cationic Indicators[a]

Fate process	**Model and model basis**	**Model result and prediction**	**Extrapolated half-life (days)**
Atmospheric oxidation (air)	AOPWIN 2010[b]	$t_{½}$ = 0.143–0.16 days	≤ 2
Ozone reaction (air)	AOPWIN 2010[b]	N/A[c]	N/A
Hydrolysis (water)	HYDROWIN 2010[b]	N/A, not in training set	N/A
Primary biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2008[b] Submodel 4: Expert Survey (qualitative results)	2.62–3.08[d] (biodegrades slowly)	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2008[b] Submodel 3: Expert Survey (qualitative results)	0.98–1.72[d] (biodegrades very slowly)	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2008[b] Submodel 5: MITI linear probability	−1.51 to −0.63[e] (biodegrades very slowly)	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2008[b] Submodel 6: MITI non-linear probability	0[e] (biodegrades very slowly)	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	DS TOPKAT c2005–2009 Probability	N/A	
Ultimate biodegradation: Biodegradation (aerobic) (water)	CATALOGIC ©2004–2011 % BOD	% BOD =7 (biodegrades very slowly)	≥ 182

Abbreviations: BOD, biological oxygen demand; MITI, Ministry of International Trade and Industry (Japan); N/A, not applicable;

[a] Substances used in this summary include the following CAS RNs: 298-83-9 and 1871-22-3..

[b] EPISuite (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.

Table A-9d. Summary of modelled data for degradation of Benzidine-based Precursors[a]

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Atmospheric oxidation (air)	AOPWIN 2010[b]	$t_{½}$ = 0.08–0.09 days	≤ 2
Ozone reaction (air)	AOPWIN 2010[b]	N/A[c]	N/A
Hydrolysis (water)	HYDROWIN 2010[b]	N/A, not in training set	N/A
Primary biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2008[b] Submodel 4: Expert Survey (qualitative results)	3.50–3.65[d] (may biodegrade fast)	≤182
Ultimate biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2008[b] Submodel 3: Expert Survey (qualitative results)	1.80–2.31[d] (biodegrades slowly)	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2008[b] Submodel 5: MITI linear probability	−0.11 to 0.11[e] (biodegrades very slowly)	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2008[b] Submodel 6: MITI non-linear probability	0–0.01[e] (biodegrades very slowly)	≥ 182
Ultimate biodegradation: Biodegradation (aerobic)	DS TOPKAT c2005–2009 Probability	NA	
Ultimate biodegradation: Biodegradation (aerobic) (water)	CATALOGIC ©2004–2011 % BOD	% BOD = 7–26 (biodegrades slowly)	≥ 182

Abbreviations: BOD, biological oxygen demand; MITI, Ministry of International Trade and Industry (Japan); N/A, not applicable;

[a] Substances used in this summary include the following CAS RNs: 91-92-9 and 93940-21-7..

[b] EPISuite (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.

Table A-9e. Summary of calculated and modelled data for degradation of Benzidine Derivatives[a]

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Atmospheric oxidation (air)	Meylan and Howard 1993[b] (calculated)	$t_{½}$ = 0.167–0.25 day (1.3×10^{-10} to 1.9×10^{-10} cm^3 molecule – sec)	≤ 2
Atmospheric oxidation (air)	AOPWIN 2010[c]	$t_{½}$ = 0.052–0.079 day	≤ 2
Ozone reaction (air)	AOPWIN 2010[c]	N/A[d]	N/A
Hydrolysis (water) (CAS RN 91-97-4)	HYDROWIN 2010[c]	$t_{½}$ = < 10 days (even at low pHs)	N/A
Primary biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2008[c] Submodel 4: Expert Survey (qualitative results)	2.925–3.433[e] “may biodegrade fast”	≤ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2008[c] Submodel 3: Expert Survey (qualitative results)	2.158–2.31[e] “biodegrades slowly”	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2008[c] Submodel 5: MITI linear probability	−0.105 to 0.111[f] “biodegrades very slowly”	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	BIOWIN 2008[c] Submodel 6: MITI non-linear probability	0.006–0.027[f] “biodegrades very slowly”	≥ 182
Ultimate biodegradation: Biodegradation (aerobic) (water)	DS TOPKAT c2005–2009 Probability	0–0.3[f] “biodegrades slowly”	≥ 182
Ultimate biodegradation:	CATALOGIC ©2004–	% BOD = 0.6–15.85	≥ 182

Biodegradation (aerobic) (water)	2011 % BOD	“biodegrades slowly”	

Abbreviations: BOD, biological oxygen demand; MITI, Ministry of International Trade and Industry (Japan); N/A, not applicable

[a] Substances used in this summary include the following CAS RNs: 119-90-4, 119-93-7 and 366-29-0. Hydrolysis predictions are also given for CAS RN 91-97-4, the only substance that is susceptible to hydrolysis.

[b] Calculated using an atmospheric concentration of 5 × 10^5 hydroxyl radicals per cubic centimetre.

[c] EPISuite (2012.

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

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

[f] Output is a probability score.

Table A-10. Empirical data for aquatic toxicity for substances in the Benzidine Derivatives subgroup

CAS RN	**Test organism**	**Type of test (duration)**	**Endpoint**	**Value (mg/L)[b]**	**Reference**
91-97-4	Rainbow trout *Oncorhynchus mykiss*	Acute (96 h)	NOEC	0.18–0.19	ECHA 2012
91-97-4	Rainbow trout *Oncorhynchus mykiss*	Acute (96 h)	LC$_{50}$	0.25	ECHA 2012
119-93-7	Alga *Pseudokircheneriella subcapitata*	Chronic (72 h)	NOEC (growth area under the curve)	0.32	MITI 2000
119-93-7	Alga	Chronic (72 h)	NOEC (growth rate)	0.45	MITI 2000
119-93-7	*Pseudokircheneriella subcapitata*	Chronic (72 h)	EC$_{50}$ (growth area under the curve)	2	MITI 2000
119-93-7	Alga	Chronic (72 h)	EC$_{50}$ (growth rate)	6.3	MITI 2000
119-93-7	*Daphnia*	Chronic (21 days)	NOEC (reproduction)	0.16	Kuhn et al. 1989
119-93-7	*Daphnia*	Acute (24 h)	EC$_0$ (behaviour)	1.5	Kuhn 1989
119-93-7	*Daphnia*	Acute (24 h)	EC$_{50}$ (behaviour)	3.2	Kuhn 1989
119-93-7	*Daphnia*	Chronic (21 days)	NOEC	0.26	MITI 2000
119-93-7	*Daphnia*	Chronic (21 days)	EC$_{50}$	0.64	MITI 2000
119-93-7	*Daphnia*	Acute (48 h)	EC$_{50}$ (immobilization)	4.5	MITI 2000
119-93-7	Fish	Acute (96 h)	LC$_{50}$	13	MITI 2000

	Oryzias latipes				
119-93-7	Fish *Oryzias latipes*	Acute (48 h)	LC$_{50}$	55.8	MITI 1992
119-93-7	Green alga *Desmodesmus subspicatus*	Chronic (72 h) (growth rate)	NOEC	≥ 1.5	ECHA 2012
119-93-7	Green alga *Desmodesmus subspicatus*	Chronic (72 h) (growth rate)	EC$_{50}$	> 1.5	ECHA 2012
119-93-7	*Daphnia magna*	Chronic (48 h)	NOEC	≥ 1.2	ECHA 2012
119-93-7	*Daphnia magna*	Chronic (48 h)	EC$_{50}$	> 1.2	ECHA 2012

Abbreviations: EC$_{50}$, the concentration of a substance that is estimated to cause some effect on 50% of the test organisms; LC$_{50}$, the concentration of a substance that is estimated to be lethal to 50% of the test organisms; NOEC, no-observed-effect concentration (the highest concentration in a toxicity test not causing a statistically significant effect in comparison with the controls)

Appendix B. Aquatic PEC Calculations for Benzidine-based Acid and Direct Dyes Used in Textile Dyeing

The method used for the stepwise estimation of the aquatic PECs from the textile wet processing sector is described as follows.

Step 1: Maximum annual quantity of the Benzidine-based Acid or Direct Dyes used by the textile wet processing sector

There are 10 acid dyes in the Benzidine-based Acid Dyes group. Survey data showed that one acid dye was reported in an annual quantity of 100–1000 kg/year, and no quantities were reported for each of the remaining nine acid dyes with the reporting threshold of 100 kg/year. The maximum annual quantity of the Benzidine-based Acid Dyes would then be 1900 kg/year by adding together the upper end of one reported quantity (1000 kg/year) and 9 times the 100 kg/year threshold.

> Maximum annual quantity of Benzidine-based Acid Dyes used by the textile sector = 1900 kg/year

There are 25 direct dyes in the Benzidine-based Direct Dyes group. Survey data showed that one direct dye was reported in an annual quantity of 0–100 kg/year, and no report was received for each of the remaining 24 direct dyes with the reporting threshold of 100 kg/year. The maximum annual quantity of the Benzidine-based Direct Dyes would then be 2500 kg/year by adding together the upper end of one reported quantity (100 kg/year) and 24 times the 100 kg/year threshold.

> Maximum annual quantity of Benzidine-based Direct Dyes used by the textile sector = 2500 kg/year

Step 2: Maximum annual quantity of the Benzidine-based Acid or Direct Dyes used at one mill

The highest quantity of the Benzidine-based Acid Dyes sold to one single textile mill was 300 kg/year according to industry surveys conducted for the years 2005 and 2006 under *Canada Gazette* notices issued pursuant to section 71 of CEPA 1999 (Canada 2006b, 2008b). This highest quantity is selected as the maximum quantity of the Benzidine-based Acid Dyes used at any given single mill. No survey data are available on the highest quantity of the Benzidine-based Direct Dyes sold to one single textile mill above the 100 kg/year reporting threshold. Thus, the maximum quantity of the Benzidine-based Direct Dyes used at any given single mill is assumed to be 100 kg/year.

Maximum annual quantity of the Benzidine-based Acid Dyes used at one mill = 300 kg/year

Maximum annual quantity of the Benzidine-based Direct Dyes used at one mill = 100 kg/year

Step 3: Daily use quantity at one mill

The daily use quantity of the Benzidine-based Acid or Direct Dyes at one mill is estimated based on a typical daily quantity of textile dyed and a typical dye use rate. Typically, a dyelot is completed within 6 hours from batch dyeing or 8 hours from continuous dyeing (US EPA 1994). When a mill operates three shifts or 24 hours/day, the maximum number of dyelots completed per day would be four dyelots, as determined for batch dyeing. One dyelot typically consists of 454 kg of textile, so the daily quantity of textile dyed would be 1816 kg/day (454 kg/dyelot × 4 dyelots/day). For a typical dye use rate of 0.02 kg dyes per kilogram of textile (Cai et al. 1999), the daily quantity of the Benzidine-based Acid or Direct Dyes used at one mill is estimated as:

Daily quantity of the Benzidine-based Acid or Direct Dyes used at one mill = 1816 kg/day × 0.02 kg/kg = 36 kg/day

Step 4: Number of annual release days from one mill

The number of annual release days from one mill is assumed to be the same as the number of the annual operation days, since the wastewater resulting from dyeing (spent bath and rinse water) is generally not stored on site and is released to municipal sewers soon after it is generated. The number of annual release days is then estimated as 8.3 days for the Benzidine-based Acid Dyes and 2.8 days for the Benzidine-based Direct Dyes by dividing the maximum annual quantity (300 kg/year for the Benzidine-based Acid Dyes or 100 kg/year for the Benzidine-based Direct Dyes) used at one mill by their daily use quantity (36 kg/day). These values represent the maximum durations for the continuous release of the Benzidine-based Acid or Direct Dyes via wastewater.

Number of annual release days from one mill for the Benzidine-based Acid Dyes = 8.3 days

Number of annual release days from one mill for the Benzidine-based Direct Dyes = 2.8 days

Step 5: Daily release to sewers from one mill

The daily release of the Benzidine-based Acid or Direct Dyes to sewers is estimated based on their respective emission factors to wastewater. On average, the emission factor is 10% for acid dyes and 12% for direct dyes (OECD 2004). The daily release to sewers of the Benzidine-based Acid or Direct Dyes from one mill is then calculated by multiplying the daily use quantity by the emission factor.

Daily release of the Benzidine-based Acid Dyes to sewers from one mill = 36 kg/day × 10% = 3.6 kg/day

Daily release of the Benzidine-based Direct Dyes to sewers from one mill = 36 kg/day × 12% = 4.3 kg/day

These release estimates are based on the assumption of zero removal for on-site wastewater treatment, because specific information is not available on the type of on-site wastewater treatment at each of the mills evaluated. The use of the zero removal assumption yields conservative release estimates.

Step 6: Estimated wastewater influent concentration

The concentration of the Benzidine-based Acid or Direct Dyes in wastewater influent is calculated by dividing the daily release quantity (3.6 kg/day for the Benzidine-based Acid Dyes or 4.3 kg/day for the Benzidine-based Direct Dyes) by the wastewater flow (L/day) of a municipal wastewater treatment system. The wastewater flow varies from location to location. For example,

Wastewater flow in Arthur, ON = 1 041 600 L/day

Wastewater flow in Montréal, QC = 2 786 797 997 L/day

The concentrations of the Benzidine-based Acid or Direct Dyes in wastewater influent at these two locations are determined as:

Wastewater influent concentration for the Benzidine-based Acid Dyes in Arthur, ON

$= 3.6 \text{ kg/day} / 1\,041\,600 \text{ L/day} = 3.46 \times 10^{-6} \text{ kg/L} = 3460\ \mu\text{g/L}$

Wastewater influent concentration for the Benzidine-based Acid Dyes in Montréal, QC

$= 3.6 \text{ kg/day} / 2\,786\,797\,997 \text{ L/day} = 1.29 \times 10^{-9} \text{ kg/L} = 1.29\ \mu\text{g/L}$

Wastewater influent concentration for the Benzidine-based Direct Dyes in Arthur, ON

= 4.3 kg/day / 1 041 600 L/day = 4.13 × 10^{-6} kg/L = 4130 μg/L

Wastewater influent concentration for the Benzidine-based Direct Dyes in Montréal, QC

= 4.3 kg/day / 2 786 797 997 L/day = 1.54 × 10^{-9} kg/L = 1.54 μg/L

Step 7: Removal by off-site wastewater treatment systems

No suitable model was available to estimate the removal of the Benzidine-based Acid or Direct Dyes through wastewater treatment systems. The models used by Environment Canada (e.g., ASTreat 2006; STP 2006) are designed for neutral substances and are not suitable for ionic chemicals. Since both Benzidine-based Acid Dyes and Benzidine-based Direct Dyes are water-soluble anionic compounds (US EPA 1996), they fall outside the domain of applicability for the above-mentioned models.

Literature data are available on the wastewater treatment removal of azo dyes in general and can be used to provide removal estimates for the Benzidine-based Acid or Direct Dyes, since they are azo dyes. In a Danish survey report (Øllgaard et al. 1998), removal rates of 40–80% were found for azo dyes. This removal range is a result of adsorption to sludge alone, without accounting for any additional removal by abiotic or biotic degradation. This range is therefore expected to occur with all three common wastewater treatment types (primary, secondary and lagoons), since all these systems provide sludge removal or settling. As an approximation, an average (60%) of this removal range is selected for the Benzidine-based Acid or Direct Dyes. The average is judged to be more statistically representative than any other value of the different wastewater treatment systems involved and the different individual azo dye substances in the Benzidine-based Acid or Direct Dyes.

Wastewater treatment removal for the Benzidine-based Acid or Direct Dyes = 60%

Step 8: Lagoon dilution

Many textile mills are located in municipalities served by lagoons. These lagoons contain large volumes of water and have long hydraulic retention times. The retention time of a lagoon is measured in weeks to months, according to field data collected through the CMP Monitoring and Surveillance Program at Environment Canada (Smyth 2012). The implication of a long retention time is that a substance entering a lagoon within a relatively short duration is subject to not only removal, but also dilution. As a result, the substance concentration in the lagoon effluent is reduced by both removal and dilution. This is the case with the release of the Benzidine-based Acid or Direct Dyes. The duration of the release within a year was estimated previously as 8.3 days for the Benzidine-based Acid Dyes or 2.8 days for the Benzidine-based Direct Dyes (see Step 4 above). These durations are short compared with a lagoon's residence time. Dilution is therefore justified. Such dilution is, however, not expected in primary or secondary treatment systems, because their hydraulic retention times are short, typically measured in hours.

No quantitative method is available to determine the degree of lagoon dilution. Nevertheless, the ratio of a lagoon's retention time to a substance's release duration can be considered as the maximum dilution, because the ratio is equivalent to the full dilution or the volume ratio of the entire lagoon water to the wastewater containing a specific substance. As an estimate, the lagoon retention time in weeks to months is interpreted as 42 days (6 weeks) to 84 days (12 weeks). The full dilution is then determined to be 5- to 10-fold for the Benzidine-based Acid Dyes or 15- to 30-fold for the Benzidine-based Direct Dyes by dividing the retention time (42–84 days) by the release duration (8.3 days for the Benzidine-based Acid Dyes or 2.8 days for the Benzidine-based Direct Dyes). As an approximation, an average is selected from each range for lagoon dilution, 7.5-fold for the Benzidine-based Acid Dyes and 22.5-fold for the Benzidine-based Direct Dyes.

Lagoon dilution for the release of the Benzidine-based Acid Dyes = 7.5

Lagoon dilution for the release of the Benzidine-based Direct Dyes = 22.5

Step 9: Wastewater effluent concentration

The concentration of the Benzidine-based Acid or Direct Dyes in wastewater effluent is determined by applying the wastewater treatment removal to the influent concentration. Dilution is also considered for lagoons. For example, the wastewater from a mill in Montréal, QC, is discharged to a primary system, and only the 60% removal is used to estimate the effluent concentration.

Wastewater effluent concentration for the Benzidine-based Acid Dyes in Montréal, QC

= influent concentration × (1 − removal)

= 1.29 µg/L × (1 − 60%) = 0.52 µg/L

Wastewater effluent concentration for the Benzidine-based Direct Dyes in Montréal, QC

= influent concentration × (1 − removal)

= 1.54 μg/L × (1 − 60%) = 0.62 μg/L

For a mill in Arthur, ON, the mill wastewater is discharged to a lagoon, and the concentration of the Benzidine-based Acid or Direct dyes in the effluent is estimated as:

Wastewater effluent concentration for the Benzidine-based Acid Dyes in Arthur, ON

= influent concentration × (1 − removal) / lagoon dilution for the Benzidine-based Acid Dyes

= 3460 μg/L × (1 − 60%) / 7.5 = 185 μg/L

Wastewater effluent concentration for the Benzidine-based Direct Dyes in Arthur, ON

= influent concentration × (1 − removal) / lagoon dilution for the Benzidine-based Direct Dyes

= 4130 μg/L × (1 − 60%) / 22.5 = 73.4 μg/L

Step 10: Predicted aquatic environmental concentration

The predicted aquatic environmental concentration (aquatic PEC) is determined by applying the receiving water dilution to the effluent concentration. Since the aquatic PEC is assessed near the discharge point, the receiving water dilution selected should also be applicable to this condition. The full dilution potential of a river is considered appropriate if it is between 1 and 10. Otherwise, the dilution is kept at 10 for both large rivers and still waters.

For the wastewater treatment system (a lagoon) in Arthur, ON, the receiving water is the Conestogo River, and its dilution potential is determined to be 7.64 (ratio of the 10th percentile river flow 7 957 160 L/day to the wastewater effluent flow 1 041 600 L/day). The aquatic PEC for the Benzidine-based Acid or Direct Dyes at the site of Arthur, ON, is then estimated as:

Aquatic PEC for the Benzidine-based Acid Dyes at site of Arthur, ON

= Wastewater effluent concentration / Receiving water dilution

= 185 μg/L / 7.64 = 24.2 μg/L

Aquatic PEC for the Benzidine-based Direct Dyes at site of Arthur, ON

= Wastewater effluent concentration / Receiving water dilution

= 73.4 µg/L / 7.64 = 9.6 µg/L

For the wastewater treatment system (primary) in Montréal, QC, the receiving water, the St. Lawrence River, has a very large flow, so the dilution is limited to 10 near the discharge point. The aquatic PEC for the Benzidine-based Acid or Direct Dyes at the site of Montréal, QC, is then estimated as:

Aquatic PEC for the Benzidine-based Acid Dyes at site of Montréal, QC

= Wastewater effluent concentration / Receiving water dilution

= 0.52 µg/L / 10 = 0.052 µg/L

Aquatic PEC for the Benzidine-based Direct Dyes at site of Montréal, QC

= Wastewater effluent concentration / Receiving water dilution

= 0.62 µg/L / 10 = 0.062 µg/L

Although there are sites where multiple textile mills are identified to discharge to one single wastewater treatment system, the chance of more than one mill at any of these sites using and releasing the same acid or direct dyes is expected to be low. This is because mills are operated year-round, while the release from one single mill occurs only for 8.3 days for the Benzidine-based Acid Dyes and 2.8 days for the Benzidine-based Direct Dyes. The release overlapping within these short periods is therefore a low possibility. As a result, the aquatic PEC resulting from each single mill can be considered to reflect the level of exposure near the discharge point, although there are two or more mills identified at a site.

The aquatic PECs calculated for the Benzidine-based Acid and Direct Dyes are summarized in Table 12 in the section on Characterization of Ecological Risk.

Appendix C. Soil PEC Calculations for Benzidine-based Acid and Direct Dyes Used in Textile Dyeing

The method used for the stepwise estimation of the soil PECs from the textile wet processing sector and biosolids application is described as follows.

Step 1: Biosolids quantity

The quantity of biosolids produced from the wastewater treatment systems at the 33 sites evaluated for the aquatic exposure can be approximately assumed to equal the quantity of sludge generated. The quantity of sludge generated can be estimated from the per capita sludge production rate and the population served by the wastewater treatment systems. The per capita sludge production rate is reported as 0.090 kg/day per person from primary treatment and 0.115 kg/day per person from secondary treatment (Droste 1997). The combined population served by the wastewater treatment systems at the 33 sites is determined to be 5 661 000 persons based on the population served by each individual treatment system. This combined population is broken down into 1 810 000 persons serviced by primary treatment and 3 851 000 persons serviced by secondary treatment. The quantity of sludge generated or the quantity of biosolids produced is then estimated as:

> Biosolids quantity = 0.090 kg/day per person × 1 810 000 persons + 0.115 kg/day per person × 3 851 000 persons = 605 765 kg/day = 221 104 000 kg/year

Step 2: Quantity of Benzidine-based Acid or Direct Dyes in biosolids

The quantity of Benzidine-based Acid or Direct Dyes in biosolids is estimated based on the maximum quantity of Benzidine-based Acid or Direct Dyes used for textile dyeing and the removal efficiency by wastewater treatment. The maximum quantity used for textile dyeing was estimated previously as 1900 kg/year for Benzidine-based Acid Dyes and 2500 kg/year for Benzidine-based Direct Dyes. The wastewater treatment removal by sludge sorption in the range of 40–80%, as reported for azo dyes by the Danish Environmental Protection Agency (Øllgaard et al. 1998), is considered applicable to both Benzidine-based Acid and Direct Dyes. An average removal rate of 60% is judged to be statistically representative of a large number of wastewater treatment operations across the sites of the 75 mills involving different treatment types and different individual azo dye substances. This removal rate is therefore used to estimate the quantity of Benzidine-based Acid or Direct Dyes in biosolids.

> Quantity of Benzidine-based Acid Dyes in biosolids = 1900 kg/year × 60% = 1140 kg/year

Quantity of Benzidine-based Direct Dyes in biosolids = 2500 kg/year × 60% = 1500 kg/year

These estimated quantities are conservative, since they are not corrected for the amounts released to lagoons. In general, lagoons do not produce biosolids, and the amounts released to lagoons therefore do not end up in biosolids.

Step 3: Concentration of Benzidine-based Acid or Direct Dyes in biosolids

The concentration of the Benzidine-based Acid or Direct Dyes in biosolids is calculated by dividing the quantity in biosolids by the quantity of biosolids produced.

Concentration of Benzidine-based Acid Dyes in biosolids

= 1140 kg/year / 221 104 000 kg/year = 0.000 005 2 kg/kg = 5.2 mg/kg

Concentration of Benzidine-based Direct Dyes in biosolids

= 1500 kg/day / 221 104 000 kg/day = 0.000 006 8 kg/kg = 6.8 mg/kg

Step 4: Land application rate

The land application rate of municipal wastewater sludge (or biosolids) is regulated by the provinces and territories. The allowable annual limits on a dry weight basis are 1.6 tonnes/ha in Ontario, 3.4 tonnes/ha in British Columbia, 4.4 tonnes/ha in Quebec and 8.3 tonnes/ha in Alberta (Crechem 2005). The limit in Alberta is the highest in Canada and is used for soil exposure calculations.

Annual land application rate = 8.3 tonnes/ha = 0.83 kg/m^2

Step 5: Quantity of Benzidine-based Acid or Direct Dyes over 10 years of biosolids application

The European Chemicals Agency (ECHA 2010) suggests using 10 consecutive years as a length of accumulation in evaluating soil exposure resulting from biosolids application. The quantity of the Benzidine-based Acid or Direct Dyes received per square metre of the amended soil during this 10-year period would be:

Quantity of Benzidine-based Acid Dyes per square metre of soil

= biosolids application rate × 10 years × concentration of Benzidine-based Acid Dyes in biosolids

= 0.83 kg/m^2 per year × 10 years × 5.2 mg/kg = 43.2 mg/m^2

Quantity of Benzidine-based Direct Dyes per square metre of soil

= biosolids application rate × 10 years × concentration of Benzidine-base Direct Dyes in biosolids

= 0.83 kg/m^2 per year × 10 years × 6.8 mg/kg = 56.4 mg/m^2

Step 6: Mass of ploughing-layer soil per square metre

The European Chemicals Agency (ECHA 2010) also suggests using 20 cm (i.e., 0.2 m) as the ploughing depth in determining a mixing layer. Using a dry soil density of 1200 kg/m^3 (Williams 1999), the mass of the top 20 cm soil layer per square metre is:

Mass of ploughing layer per 1 m^2 = 1200 kg/m^3 × 1 m^2 × 0.2 m = 240 kg/m^2

Step 7: Soil PEC

The soil PEC is determined by dividing the quantity of the Benzidine-based Acid or Direct Dyes upon 10-year land application by the mass of ploughing-layer soil on a per square metre basis.

Soil PEC for Benzidine-based Acid Dyes = 43.2 mg/m^2 / 240 kg/m^2 = 0.18 mg/kg

Soil PEC for Benzidine-based Direct Dyes = 56.4 mg/m^2 / 240 kg/m^2 = 0.24 mg/kg

Appendix D. Estimated Exposures to 3,3′-DMB from Polyamide Cooking Utensils

Exposures to 3,3′-DMB from use of black polyamide cooking utensils were estimated, based on information indicating that this substance can leach from the utensil to soup or sauce during use. Estimated exposures are based on the following assumptions: that an individual uses a polyamide black cooking utensil every day, that the leaching of 3,3′-DMB remains constant over multiple uses and that the utensil remains in the hot soup or sauce (while cooking) for a long period of time. Estimated daily intakes were derived using a detailed intake of foods (Health Canada 1998) and the median leaching level of 3,3′-DMB (based on the third extraction levels, using the LOD for non-detect utensils and an average volume:area ratio when not indicated) calculated from the Danish study (McCall et al. 2012).

Estimates are considered to be conservative, as leaching test conditions (3% volume per volume [v/v] aqueous acetic acid, 100°C, 30 minutes to 4 hours) are not truly representative of real use conditions; it is unlikely that all soups or sauces will be stirred continually for the entire duration of this length of time or at this temperature. As shown in the study, the concentration leaching out of these utensils is highly variable.

Estimated intake from a food item = [Chemical in food (µg/g) × Consumption (g/day)] / Body weight

3,3′-DMB in food (median leaching level):

3,3′-DMB in food = 1.4 µg/kg

Body weights (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

Conservative estimates of daily intakes of 3,3′-DMB from use of black polyamide cooking utensils are presented in Table D-1.

Table D-1. Consumption and estimated daily intakes of 3,3′-DMB from use of black polyamide cooking utensils

(a) 0–4 years

Food item	0–6 months: Consumption (g/day)	0–6 months: Intake (µg/kg-bw per day)	0.5–4 years: Consumption (g/day)	0.5–4 years: Intake (µg/kg-bw per day)
Soups, meat, canned	5.36	0.0010	41.64	0.0037
Soups, vegetable	4.97	0.0009	8.16	0.0007
Soups, tomato	1.91	0.0004	6.50	0.0006
Soups, dehydrated	0.33	0.0001	10.43	0.0009
Sauces and gravies	0.68	0.0001	5.64	0.0005
Total	13.24	0.0025	72.38	0.0065

(b) 5–19 years

Food item	5–11 years: Consumption (g/day)	5–11 years: Intake (µg/kg-bw per day)	12–19 years: Consumption (g/day)	12–19 years: Intake (µg/kg-bw per day)
Soups, meat, canned	41.76	0.0019	35.12	0.0008
Soups, vegetable	10.99	0.0005	21.88	0.0005
Soups, tomato	11.67	0.0005	6.95	0.0002
Soups, dehydrated	7.98	0.0004	7.91	0.0002
Sauces and gravies	8.98	0.0004	14.29	0.0003
Total	81.38	0.0036	86.15	0.0020

(c) 20–60+ years

Food item	20–59 years: Consumption (g/day)	20–59 years: Intake (µg/kg-bw per day)	60+ years: Consumption (g/day)	60+ years: Intake (µg/kg-bw per day)
Soups, meat, canned	55.29	0.0011	54.16	0.0010

Food item	20–59 years: Consumption (g/day)	20–59 years: Intake (µg/kg-bw per day)	60+ years: Consumption (g/day)	60+ years: Intake (µg/kg-bw per day)
Soups, vegetable	15.03	0.0003	18.17	0.0004
Soups, tomato	6.92	0.0001	7.93	0.0002
Soups, dehydrated	8.33	0.0002	5.70	0.0001
Sauces and gravies	14.82	0.0003	10.76	0.0002
Total	100.40	0.0020	96.72	0.0019

Appendix E. Estimates of Exposure to Acid Red 97 from Textile and Leather Products

Table E-1. Estimated upper-bounding exposures to Acid Red 97 via contact with textile materials

Product scenario	Daily exposure (mg/kg-bw per day)
Textiles; personal apparel (adult; dermal)	0.002 6
Textiles; baby sleeper (infant; dermal)	0.004 0
Textiles (infant; oral)	2.7×10^{-5}

Dermal Exposure from Textile

Exposure estimate = $\frac{SA \times AW \times SCF \times C \times M \times DA \times F \times P}{BW}$

Dermal exposure was estimated based on a scenario of full (100%) body coverage from wearing clothing to account for exposures from multiple pieces of apparel that cover the entire surface area of the body.

Oral Exposure from Textile

Exposure estimate = $\frac{SA \times AW \times SCF \times C \times M \times F \times P}{BW}$

Oral exposure to Acid Red 97 is estimated based on a scenario assuming that the infant is mouthing a textile object (e.g., blanket, textile toy) that may release Acid Red 97.

Parameters

***SA*: Total surface area** = 18 200 cm^2 (dermal; adult; personal apparel) and 3020 cm^2 (dermal; infant; baby sleeper) (Health Canada 1998); 20 cm^2 (oral; infant Zeilmaker et al. 2000).

***AW*: Area weight of textile** = 20 mg/cm^2 (US EPA 2012).

***SCF*: Skin contact factor** = 1.

***C*: Concentration** = 0.01 (unitless) (BfR 2007). Based on the default model developed by the “Textiles” Working Group established at the German Federal Institute for Risk Assessment (BfR 2007), assuming that a standard textile garment of 100 g/m^2 is dyed with 1% active dye ingredient.

***M*: Migration fraction** = 0.0005 (BfR 2007). The migration of azo dyes from textiles varies considerably depending on the type of fibre, the type of dye used, the dye load, dyeing technology and colour intensity and after treatment. The exposure from textiles is partly dictated by the amount of dye that migrates from textile material onto human skin (ETAD 1983) or via mouthing. The “Textiles” Working Group (BfR 2007) uses a peak initial migration of 0.5% to estimate exposure to dyes from newly bought unwashed garments, and the chronic migration rate is assumed to be one tenth of the value measured for the first migration to reflect exposure after initial washes. It is assumed that the sweat migration rate is similar to the salivary migration rate; this is consistent with observations of leaching behaviours of dyes from textiles reported by Zeilmaker et al. (1999). Accordingly, the fraction of dye that migrates from a textile material per wear is assumed to be 0.0005 for both dermal and oral exposure.

***DA*: Dermal absorption** = 100%.

***F*: Frequency** = 1×/day.

***P*: Probability that Acid Red 97 is present in textiles** = 10%. In the RIVM risk assessment of azo dyes and aromatic amines from garments and footwear (Zeilmaker et al. 1999), the authors derived a chance of 8% for the appearance of carcinogenic azo dyes and aromatic amines in garments based on four European studies. The congener of Acid Red 97 is not an EU22 amine; the prevalence of this dye is not clear because there is limited product testing and monitoring on non-EU22 amines and associated dyes. From the limited data available (Danish EPA 1998; Brüschweiler et al. 2014), the detection of most non-EU22 amines in textiles is usually less than 10%. Accordingly, the presence of associated dyes in textiles would be the same or lower. The chances of an individual’s outfit containing Acid Red 97 every day are low. Given the conservatism used in other parameters in this exposure scenario (e.g. full body coverage), the probability that Acid Red 97 is present in a textile is assumed to be 10% in this screening assessment based on professional judgement.

***BW*: Body weight** = 7.5 kg for infant, 70.9 kg for adult (Health Canada 1998).

Table E-2. Estimated upper-bounding exposures to Acid Red 97 from dermal contact with leather products

Product scenario	Per event exposure (mg/kg-bw)
Shoes	5.8×10^{-2}
Boots	1.9×10^{-2}
Gloves	2.1×10^{-3}
Jackets and coats	7.7×10^{-2}
Trousers	5.0×10^{-2}
Furniture	2.3×10^{-2}
Toys	4.0×10^{-2}

Dermal Exposure from Leather

Exposure estimate = $\frac{SA \times AW \times SCF \times C \times M \times DA}{BW}$

Direct skin contact with articles of leather can result in dermal exposure to dyes used in leather dyeing. Of all the leather products considered, the potential drivers for exposure are presented below; furniture, apparel (e.g., jackets, trousers and gloves), footwear (e.g., shoes and boots) and toys, where it is assumed that direct contact with the infant's palms can occur when playing with the toy. The exposure estimates presented below are considered upper-bounding based on conservative assumptions as well as not taking into account of a final application of a polyurethane sealant coating which would further reduce the consumer's dermal exposure to the leather dye.

Parameters

***SA*: Surface area of skin contact** (Health Canada 1998; Therapeutic Guidelines Ltd. 2008)

- Shoes: 1275 cm^2 (adult feet)
- Boots: 4185 cm^2 (adult legs and feet)
- Gloves: 455 cm^2 (adult hands)
- Jackets and coats: 8920 cm^2 (adult trunk and arms)
- Trousers: 5820 cm^2 (adult lower body)
- Furniture: 5005 cm^2 (adult back, buttocks and back of thighs)
- Toys: 92.5 cm^2 (infant palms)

***AW*: Area weight of leather** = 0.15 g/cm^2 (Danish EPA 2012)

***SCF*: Skin contact factor**

- Shoes: 1
- Boots: 0.1
- Gloves: 0.1
- Jackets and coats: 0.19
- Trousers: 0.19
- Furniture: 0.1
- Toys: 1

When the entire leather article is in direct contact with the skin, SCF is assumed to be 1. When the leather article is in indirect contact with the skin (e.g., shielding due to interior lining), SCF is assumed to be 0.1, which is a default value used to account for exposure due to diffusion of sweat-extracted dye from the leather material through the shielding fabric onto the skin (Zeilmaker et al. 1999). When a portion of the leather article is in direct contact and the remaining portion is in indirect contact, a weighted SCF is calculated: $[(SA_{direct} \times 1) + (SA_{indirect} \times 0.1)]/(SA_{total})$.

***C*: Concentration** = 0.02 (unitless weight fraction) (Øllgaard et al. 1998)

***M*: Migration fraction** = 0.1% (i.e., 39% over 365 days).

The dermal exposure to dyes from leather is partly dictated by the amount of dye that migrates from leather material onto human skin. Zeilmaker et al. (1999) measured the experimental leaching of azo dyes from leather footwear material to be 15% and 39%. The leaching was determined by extracting from 1 g of unwashed material from the upper side of a newly bought leather shoe with 100 mL sweat stimulant (extraction conditions: 16 hours at 37°C while shaking). These extraction conditions are expected to overestimate the migration of dyes from sweat. In estimating exposure to dyes from leather articles, it is assumed that 39% of the dye content leaches over one year and is available for dermal exposure, which would be equivalent to 0.1% leaching in one day.

***DA*: Dermal absorption** = 100%.

***BW*: Body weight** = 7.5 kg for infant, 70.9 kg for adult (Health Canada 1998).

Appendix F. Benchmark Dose Calculations for 3,3′-DMOB·2HCl

Table F-1. Incidences of tumours in F344/N rats exposed to 3,3′-DMOB·2HCl (CAS RN 20325-40-0) in drinking water (NTP 1990)[a]

Tumours	0 ppm	80 ppm	170 ppm	330 ppm
Equivalent dose for male rats (mg/kg-bw per day)	0	6	12	21
Skin basal cell or sebaceous gland neoplasms	2/59	33/44	56/72	41/56
Skin squamous cell neoplasms	0/59	13/42	28/65	22/48
Zymbal gland neoplasms	0/58	10/45	25/75	30/60
Preputial gland adenoma or carcinoma	16/59	12/42	33/73	29/59
Oral papilloma or carcinoma	1/59	8/44	10/73	11/57
Small intestine neoplasms	0/59	4/44	7/75	5/60
Large intestine neoplasms	0/59	1/44	8/73	8/57
Liver neoplasms	1/58	4/39	7/54	8/35
Mesothelium	2/59	1/44	7/72	6/56
Equivalent dose for female (mg/kg-bw per day)	0	7	14	23
Zymbal gland neoplasms	1/60	12/45	21/74	16/59
Clitoral gland neoplasms	7/58	27/44	48/74	41/55
Mammary gland adenocarcinomas	1/60	2/45	14/73	20/57

[a] Data collected here represent effective rate, because of mortality at higher treatment doses. Effective rate: number of tumour-bearing animals/effective number of animals, i.e., number of animals alive at the first occurrence of this tumour type in any of the groups.

Table F-2. BMD_{10} and $BMDL_{10}$ calculations (mg/kg-bw per day) for neoplasms induced by 3,3′-DMOB·2HCl in male (MR) and female (FR) F344/N rats[a]

Tumours	Model name	# of groups	AIC	*P*-value	SRI	BMR	BMD	BMDL
MR - Skin basal cell or sebaceous gland neoplasms[b]	LogLogistic	3	148.6	0.235	−0.015	0.1	0.32	0.22
MR - Skin squamous cell neoplasms	Multistage	4	211.2	0.518	0	0.1	1.96	1.49
MR - Zymbal gland neoplasms	Multistage cancer	4	225.6	0.952	0	0.1	2.98	2.44
MR - Preputial gland neoplasms	Multistage cancer	4	306.7	0.572	−0.77	0.1	5.47	3.47
MR - Oral cavity neoplasms	LogLogistic	4	174.1	0.097	−0.38	0.1	9.06	5.82
MR - Small	LogLogistic	4	113.35	0.258	0.38	0.1	15.08	9.99

Tumours	Model name	# of groups	AIC	*P*-value	SRI	BMR	BMD	BMDL
intestine neoplasms								
MR - Large intestine neoplasms	Quantal-linear	4	109.3	0.811	0.63	0.1	13.63	9.37
MR - Liver neoplasms	LogLogistic	4	119.4	0.880	−0.37	0.1	8.95	5.66
MR - Mesothelium	Quantal-linear	4	116.55	0.528	−0.14	0.1	24.36	13.14
FR - Zymbal gland neoplasms	LogLogistic	4	229.4	0.045	1.9	0.1	4.74	3.44
FR - Clitoral gland neoplasms	LogLogistic	4	265.5	0.414	−0.11	0.1	0.91	0.66
FR - Mammary gland adenocarinomas	LogProbit	4	177.9	0.692	0.26	0.1	10.70	8.21

Abbreviations: AIC, Akaike's Information Criterion; BMR, benchmark response; SRI, scaled residual of interest

[a] A dichotomous restricted model type was chosen for the BMD and BMDL analysis of cancer endpoints. Nine models were applied for analysis of each tumour data set. These models included Gamma, Logistic, LogLogistic, LogProbit, Multistage, Multistage cancer, Probit, Weibull and Quantal-linear. The best-fit model is selected from nine models for each tumour site based on the highest *P*-value of goodness of fit and the lowest AIC value (a measure of information loss from a dose–response model that can be used to compare a set of models). Generally the *P*-value of goodness of fit should be > 0.1, and the absolute value of SRI (represents observed minus predicted response divided by standard errors) should be < 2. Plots are regularly checked for visual overall fit.

[b] The highest-dose data point was deleted for this BMD and BMDL calculation. When the full data points were used for modelling, the *P*-value of goodness of fit was low (< 0.01) and did not meet the goodness-of-fit criterion (> 0.1). After the highest dose was removed, the *P*-value significantly increased (*P* = 0.235). The highest dose group was deleted because the high mortality was not relevant for fitting the dose–response at lower doses.

Appendix G. Benchmark Dose Calculations for 3,3′-DMB·2HCl

Table G-1. Incidences of tumours in F344/N rats exposed to 3,3′-DMB·2HCl (CAS RN 612-82-8) in drinking water (NTP 1991b)[a]

Tumours	0 ppm	30 ppm	70 ppm	150 ppm
Equivalent dose for male rats (mg/kg-bw per day)	0	1.8	4.0	11.2
Skin basal cell neoplasms	0/60	11/44	54/72	30/45
Skin sebaceous cell adenoma	0/60	0/44	7/72	5/49
Skin keratoacanthomas	1/60	1/44	8/67	5/27
Skin squamous cell neoplasms	0/60	2/45	17/74	27/59
Zymbal gland neoplasms	1/60	3/45	32/74	36/60
Preputial gland neoplasms	2/60	4/44	6/72	9/49
Liver neoplasms	0/60	0/45	35/72	33/55
Oral cavity neoplasms	0/60	0/44	4/67	5/32
Small intestine neoplasms	0/60	0/45	4/74	8/59
Large intestine neoplasms	0/60	0/45	6/67	15/38
Lung neoplasms	1/60	0/45	8/73	6/57
Equivalent dose for female (mg/kg-bw per day)	0	3.0	6.9	12.9
Skin basal cell neoplasms	0/60	3/45	10/69	9/46
Skin squamous cell neoplasms	0/60	3/45	9/72	12/55
Zymbal gland neoplasms	0/60	6/45	32/74	42/59
Clitoral gland neoplasms	0/60	14/45	42/73	32/58
Oral cavity neoplasms	0/60	3/45	9/73	13/59
Small intestine neoplasms	0/60	1/45	3/72	5/57
Large intestine neoplasms	0/60	1/45	7/70	4/46

[a] Data collected here represent effective rate, because of mortality at higher treatment doses. Effective rate: number of tumour-bearing animals/effective number of animals, i.e., number of animals alive at the first occurrence of this tumour type in any of the groups.

Table G-2. BMD$_{10}$ and BMDL$_{10}$ calculations (mg/kg-bw per day) for neoplasms induced by 3,3′-DMB·2HCl in male (MR) and female (FR) F344/N rats[a]

Tumours	Model name	# of groups	AIC	*P*-value	SRI	BMR	BMD	BMDL
MR - Skin basal cell neoplasms[b]	Multistage	3	134.5	1	0	0.1	1.07	0.51
MR - Skin sebaceous cell adenoma	LogLogistic	4	85.17	0.24	−0.78	0.1	7.60	4.74
MR - Skin keratoacanthomas	Multistage	4	100.2	0.48	0.81	0.1	5.24	3.24
MR - Skin squamous cell neoplasms	Quantal-linear	4	181.6	0.62	1.15	0.1	1.91	1.51
MR - Preputial gland neoplasms	LogLogistic	4	137.0	0.723	−0.35	0.1	7.11	3.87
MR - Oral cavity neoplasms	Quantal-linear	4	62.3	0.748	0.267	0.1	7.83	4.74

Tumours	Model name	# of groups	AIC	*P*-value	SRI	BMR	BMD	BMDL
MR - Small intestine neoplasms	Quantal-linear	4	82.0	0.777	0.18	0.1	8.64	5.56
MR - Large intestine neoplasms	LogProbit	4	96.4	0.732	0.439	0.1	4.57	3.45
FR - Skin basal cell neoplasms	LogLogistic	4	126.9	0.961	0.33	0.1	5.06	3.50
FR - Skin squamous cell neoplasms	LogLogistic	4	136.0	0.998	−0.11	0.1	5.16	3.62
FR - Zymbal gland neoplasms	LogLogistic	4	211.4	0.999	−0.023	0.1	2.51	1.56
FR - Clitoral gland neoplasms	LogLogistic	4	241.2	0.239	0	0.1	0.76	0.59
FR - Oral cavity neoplasms	LogLogistic	4	140.8	0.996	−0.15	0.1	5.16	3.64
FR - Small intestine neoplasms	Quantal-linear	4	70.5	0.997	0.1	0.1	15.48	9.37
FR - Large intestine neoplasms	LogLogistic	4	85.9	0.645	0.75	0.1	10.18	6.39

Abbreviations: AIC, Akaike's Information Criterion; BMR, benchmark response; SRI, scaled residual of interest

[a] A dichotomous restricted model type was chosen for the BMD and BMDL analysis of cancer endpoints. Nine models were applied for analysis of each tumour data set. These models included Gamma, Logistic, LogLogistic, LogProbit, Multistage, Multistage cancer, Probit, Weibull and Quantal-linear. The best-fit model is selected from nine models for each tumour site based on the highest *P*-value of goodness of fit and the lowest AIC value (a measure of information loss from a dose–response model that can be used to compare a set of models). Generally, the *P*-value of goodness of fit should be > 0.1, and the absolute value of SRI (represents observed minus predicted response divided by standard errors) should be < 2. Plots are regularly checked for visual overall fit. Tumour sites with low *P*-values were removed (male Zymbal gland, liver and lung).

[b] The highest-dose data point was deleted for this BMD and BMDL calculation. When the full data points were used for modelling, the *P*-value of goodness of fit was low (< 0.001) and did not meet the goodness-of-fit criterion (> 0.1). After the highest dose was removed, the *P*-value significantly increased (*P* = 1). The highest dose group was not included in the calculation because the high mortality was not relevant for fitting the dose–response at lower doses

Appendix H. Benzidine Derivatives and Benzidine-based Substances with Human Health Effects of Concern

Some of the Benzidine Derivatives, Benzidine-based Acid Dyes, Benzidine-based Direct Dyes, and Benzidine-based Precursors in this assessment have human health 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 of the above lines of evidence apply.

Table H-1. Substances with human health effects of concern based on potential carcinogenicity

Substance Name/ acronym and CAS RN	Classification for carcinogenicity[a]	Evidence of carcino-genicity from animal studies and/or human epidemiology	Release of EU22 aromatic amine by azo bond cleavage[b]	Read-across
Acid Red 128 6548-30-7	EU Category 1B carcinogen[c], NTP “Reasonably anticipated to be a human carcinogen”[c]		3,3′-DMOB	
Acid Red 114 6459-94-5	IARC 2B, EU Category 1B carcinogen[c], NTP “Reasonably anticipated to be a human carcinogen”[c]	x	3,3′-DMB	

Substance Name/ acronym and CAS RN	Classification for carcinogenicity[a]	Evidence of carcino-genicity from animal studies and/or human epidemiology	Release of EU22 aromatic amine by azo bond cleavage[b]	Read-across
Acid Black 209 68318-35-4	EU Category 1B carcinogen[c], NTP "Reasonably anticipated to be a human carcinogen"[c]		3,3′-DMB	
NAAHD 68400-36-2	EU Category 1B carcinogen[c], NTP "Reasonably anticipated to be a human carcinogen"[c]		3,3′-DMB	
Acid Red 99 3701-40-4				release 2,2′-DMB by azo bond cleavage[d]
BADB 89923-60-4				release 2,2′-DMB by azo bond cleavage[d]
Direct Red 28 573-58-0	IARC 1[c], EU Category 1B carcinogen[c], NTP "Known to be a human carcinogen"[c]		Benzidine	
Direct Brown 95 16071-86-6	IARC 1[c], EU Category 1B carcinogen[c], NTP "Known to be a human carcinogen"[c]	x	Benzidine	
Direct Blue 8 2429-71-2	EU Category 1B carcinogen[c], NTP "Reasonably anticipated to be a human carcinogen"[c]		3,3′-DMOB	

Substance Name/ acronym and CAS RN	Classification for carcinogenicity[a]	Evidence of carcino-genicity from animal studies and/or human epidemiology	Release of EU22 aromatic amine by azo bond cleavage[b]	Read-across
Direct Blue 15 2429-74-5	IARC 2B, EU Category 1B carcinogen[c], NTP “Reasonably anticipated to be a human carcinogen”[c]	x	3,3′-DMOB	
Direct Blue 151 6449-35-0	EU Category 1B carcinogen[c], NTP “Reasonably anticipated to be a human carcinogen”[c]		3,3′-DMOB	
NAAH·3Li 67923-89-1	EU Category 1B carcinogen[c], NTP “Reasonably anticipated to be a human carcinogen”[c]		3,3′-DMOB	
BABHS 70210-28-5	EU Category 1B carcinogen[c], NTP “Reasonably anticipated to be a human carcinogen”[c]		3,3′-DMOB	
NADB·4Li 71550-22-6	EU Category 1B carcinogen[c], NTP “Reasonably anticipated to be a human carcinogen”[c]		3,3′-DMOB	
NADB·Li·3Na 75659-72-2	EU Category 1B carcinogen, NTP “Reasonably anticipated to be a human carcinogen”		3,3′-DMOB	
NADB·2Li·2Na 75659-73-3	EU Category 1B carcinogen[c], NTP “Reasonably anticipated to be a human carcinogen”[c]		3,3′-DMOB	

Substance Name/ acronym and CAS RN	Classification for carcinogenicity[a]	Evidence of carcino-genicity from animal studies and/or human epidemiology	Release of EU22 aromatic amine by azo bond cleavage[b]	Read-across
NAAH·Li·2Na 75673-18-6	EU Category 1B carcinogen[c], NTP “Reasonably anticipated to be a human carcinogen”[c]		3,3′-DMOB	
NAAH·2Li·Na 75673-19-7	EU Category 1B carcinogen[c], NTP “Reasonably anticipated to be a human carcinogen”[c]		3,3′-DMOB	
NADB·2Li 75673-34-6	EU Category 1B carcinogen[c], NTP “Reasonably anticipated to be a human carcinogen”[c]		3,3′-DMOB	
NADB·Li·Na 75673-35-7	EU Category 1B carcinogen[c], NTP “Reasonably anticipated to be a human carcinogen”[c]		3,3′-DMOB	
NADB·3Li·Na 75752-17-9	EU Category 1B carcinogen[c], NTP “Reasonably anticipated to be a human carcinogen”[c]		3,3′-DMOB	
Direct Blue 14 72-57-1	IARC 2B, EU Category 1B carcinogen[c], NTP “Reasonably anticipated to be a human carcinogen”[c]	x	3,3′-DMB	
Direct Red 2 992-59-6	EU Category 1B carcinogen[c], NTP “Reasonably anticipated to be a human carcinogen”[c]		3,3′-DMB	

Substance Name/ acronym and CAS RN	**Classification for carcinogenicity[a]**	**Evidence of carcino-genicity from animal studies and/or human epidemiology**	**Release of EU22 aromatic amine by azo bond cleavage[b]**	**Read-across**
Direct Blue 25 2150-54-1	EU Category 1B carcinogen[c], NTP “Reasonably anticipated to be a human carcinogen”[c]		3,3′-DMB	
Direct Violet 28 6420-06-0	EU Category 1B carcinogen[c], NTP “Reasonably anticipated to be a human carcinogen”[c]		3,3′-DMB	
Direct Blue 295 6420-22-0	EU Category 1B carcinogen[c], NTP “Reasonably anticipated to be a human carcinogen”[c]		3,3′-DMB	
Direct Red 46 6548-29-4			3,3′-DCB	
BAHSD 71215-83-3				release 2,2′-DCB by azo bond cleavage[d]
TCDB 93940-21-7	EU Category 1B carcinogen[c], NTP “Reasonably anticipated to be a human carcinogen”[c]		3,3′-DMOB	
3,3′-DMOB 119-90-4	IARC 2B, EU Category 1B carcinogen, NTP “Reasonably anticipated to be a human carcinogen	x	N/A (EU22)	

Substance Name/ acronym and CAS RN	**Classification for carcinogenicity**[a]	**Evidence of carcino-genicity from animal studies and/or human epidemiology**	**Release of EU22 aromatic amine by azo bond cleavage**[b]	**Read-across**
3,3′-DMB 119-93-7	IARC 2B, EU Category 1B carcinogen, NTP “Reasonably anticipated to be a human carcinogen”	x	N/A (EU22)	
3,3′-DMB-2HCl[e] 612-82-8	NTP “Reasonably anticipated to be a human carcinogen”	x	N/A (HCl salt of EU22)	

[a] Classifications used for carcinogenicity are described in Environment Canada, Health Canada 2014.

[b] For this assessment, the specific EU22 aromatic amines include benzidine and three benzidine derivatives (3,3’-DMB, 3,3’-DMOB, 3,3’-DCB).

[c] Classification is not substance-specific but includes any dyes that can metabolize to benzidine, 3,3’-DMB or 3,3’-DMOB.

[d] It is considered that the benzidine derivatives 2,2’-DMB and 2,2’-DCB may be oxidized to active intermediates through the same pathway as for benzidine, 3,3’-DMB, 3,3’-DMOB and 3,3’-DCB.

[e] Classifications of 3,3’-DMB are considered to include its HCl and other common salts.