Screening Assessment

Aromatic Azo and Benzidine-based Substance Grouping

Certain Azo Direct Dyes and Azo Reactive Dyes

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 61 azo direct dyes and eight azo reactive dyes. These 69 substances constitute two subgroups of the Aromatic Azo and Benzidine-based Substance Grouping being assessed as part of the Substance Groupings Initiative of the Government of Canada's Chemicals Management Plan based on structural similarity and applications. Substances in this Grouping were identified as priorities for assessment as they met categorization criteria under subsection 73(1) of CEPA 1999 and/or were considered as a priority based on other human health concerns.

The Chemical Abstracts Service Registry Number (CAS RN)[1], *Domestic Substances List* (DSL) name and Colour Index (C.I) generic name of the 69 substances are presented in the following tables, by subgroup.

Identity of the 61 Azo Direct Dyes in the Aromatic Azo and Benzidine-based Substance Grouping

CAS RN	DSL name	Colour Index name
1325-37-7[a,b]	C.I. Direct Yellow 11	Direct Yellow 11
1325-54-8[a]	Benzenesulfonic acid, 2,2′-(1,2-ethenediyl)bis-5-nitro-, disodium salt, reaction products with 4-[(4-aminophenyl)azo]benzenesulfonic acid, sodium salts	Direct Orange 39
2829-42-7	Benzoic acid, 3,3′-[carbonylbis(imino-4,1-phenyleneazo)]bis[6-hydroxy-, disodium salt	Direct Yellow 26
2870-32-8	Benzenesulfonic acid, 2,2′-(1,2-ethenediyl)bis[5-[(4-ethoxyphenyl)azo]-, disodium salt	Direct Yellow 12
3214-47-9	1,5-Naphthalenedisulfonic acid, 3,3′-[carbonylbis[imino(2-methyl-4,1-phenylene)azo]]bis-, tetrasodium salt	Direct Yellow 50
3626-36-6	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-(phenylazo)-, disodium salt	Direct Orange 26
3687-80-7	1-Naphthalenesulfonic acid, 4-[[1-hydroxy-6-[[[[5-hydroxy-6-[(2-methoxyphenyl)azo]-7-sulfo-2-	Direct Red 26

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CAS RN	**DSL name**	**Colour Index name**
	naphthalenyl]amino]carbonyl]amino]-3-sulfo-2-naphthalenyl]azo]-, trisodium salt	
4399-55-7	1,5-Naphthalenedisulfonic acid, 3-[[4-[[4-[(6-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-6-sulfo-1-naphthalenyl]azo]-1-naphthalenyl]azo]-, tetrasodium salt	Direct Blue 71
5001-72-9	2-Naphthalenesulfonic acid, 7,7′-iminobis[4-hydroxy-3-(phenylazo)-, disodium salt	Direct Red 31
5489-77-0	2-Naphthalenesulfonic acid, 3-[[4-[(2,4-dimethyl-6-sulfophenyl)azo]-2-methoxy-5-methylphenyl]azo]-4-hydroxy-7-(phenylamino)-, disodium salt	Direct Violet 51
6406-87-7	2-Naphthalenesulfonic acid, 5-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-8-[[4-(phenylazo)-7-sulfo-1-naphthalenyl]azo]-, trisodium salt	NA
6420-33-3	1,5-Naphthalenedisulfonic acid, 3,3′-[carbonylbis[imino(5-methoxy-2-methyl-4,1-phenylene)azo]]bis-, tetrasodium salt	Direct Yellow 34
6420-41-3	2-Naphthalenesulfonic acid, 4-hydroxy-7-[[[[5-hydroxy-6-(phenylazo)-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-3-[(6-sulfo-2-naphthalenyl)azo]-, trisodium salt	Direct Red 4
6420-43-5	2-Naphthalenesulfonic acid, 4-hydroxy-7-[[[[5-hydroxy-6-[(2-methylphenyl)azo]-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-3-[(2-methyl-4-sulfophenyl)azo]-, trisodium salt	Direct Red 62
6471-09-6	Benzoic acid, 5-[[4-[[4-[[4-[(4-amino-9,10-dihydro-9,10-dioxo-3-sulfo-1-anthracenyl)amino]-2-sulfophenyl]amino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]phenyl]azo]-2-hydroxy-, trisodium salt	Direct Green 28
6476-10-4	2-Naphthalenesulfonic acid, 8-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-5-[[4-(phenylazo)-6-sulfo-1-naphthalenyl]azo]-, trisodium salt	NA
10114-47-3	7-Benzothiazolesulfonic acid, 2,2′-(azodi-4,1-phenylene)bis[6-methyl-, disodium salt	Direct Yellow 28
10134-33-5	2-Naphthalenesulfonic acid, 8-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-5-[[4-(phenylazo)-7-sulfo-1-naphthalenyl]azo]-, trisodium salt	Direct Black 56
10482-42-5	2-Naphthalenesulfonic acid, 5-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-8-[[4-(phenylazo)-6-sulfo-1-naphthalenyl]azo]-, trisodium salt	NA
12217-64-0	1,3-Naphthalenedisulfonic acid, 7,7′-[carbonylbis[imino(5-methoxy-2-methyl-4,1-phenylene)azo]]bis-, tetrasodium salt	Direct Orange 72

CAS RN	**DSL name**	**Colour Index name**
28706-21-0	1,3-Naphthalenedisulfonic acid, 7,7′-[iminobis[carbonyl(2-methyl-4,1-phenylene)azo]]bis-, tetrasodium salt	NA
32829-81-5	Benzenesulfonic acid, 2,2′-(1,2-ethenediyl)bis[5-[[4-[(4-sulfophenyl)azo]phenyl]azo]-, tetrasodium salt	NA
38801-08-0	Benzoic acid, 4,4′-[carbonylbis[imino(1-hydroxy-3-sulfo-6,2-naphthalenediyl)azo]]bis-, compd. with 2,2′,2″-nitrilotris[ethanol] (1:4)	NA
53523-90-3	Benzoic acid, 3,3′-[1,2-ethenediylbis[(3-sulfo-4,1-phenylene)azo]]bis[6-hydroxy-5-methyl-, tetralithium salt	NA
65150-80-3[a]	C.I. Direct Yellow 11, lithium salt	Direct Yellow 11 lithium salt
71033-21-1[a]	Benzothiazolesulfonic acid, 2,2′-(azodi-4,1-phenylene)bis[6-methyl-, disodium salt	NA
71767-19-6	2-Naphthalenesulfonic acid, 5-[[6-amino-1-hydroxy-3-sulfo-5-[(3-sulfophenyl)azo]-2-naphthalenyl]azo]-6-methoxy-8-[[7-sulfo-4-[(3-sulfophenyl)azo]-1-naphthalenyl]azo]-, pentasodium salt	NA
71873-49-9	Benzoic acid, 4,4′-[1,2-ethenediylbis[(3-sulfo-4,1-phenylene)-ONN-azoxy-4,1-phenyleneazo]]bis-, tetrasodium salt	NA
72139-21-0	Benzoic acid, 3,3′-[(1,4-dioxo-2-butene-1,4-diyl)bis(imino-4,1-phenyleneazo)]bis[6-hydroxy-, disodium salt	NA
72152-50-2	Benzoic acid, 2-[[6-[[4-[[6-(benzoylamino)-1-hydroxy-3-sulfo-2-naphthalenyl]azo]-3-methylbenzoyl]amino]-1-hydroxy-3-sulfo-2-naphthalenyl]azo]-, trisodium salt	NA
72245-49-9	Benzoic acid, 4-[[1-hydroxy-6-[[[[5-hydroxy-6-[(2-methyl-4-sulfophenyl)azo]-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-3-sulfo-2-naphthalenyl]azo]-, sodium salt	NA
72245-56-8	2,7-Naphthalenedisulfonic acid, 4-amino-3-[[4-[[[4-[(2,4-diaminophenyl)azo]phenyl]amino]carbonyl]phenyl]azo]-5-hydroxy-6-(phenylazo)-, sodium salt	NA
72749-87-2	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-[(2-methylphenyl)azo]-, disodium salt	NA
72749-88-3	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-[(2-methoxyphenyl)azo]-, disodium salt	NA
72869-93-3	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-[(6-sulfo-2-naphthalenyl)azo]-, compd. with 2,2′-(methylimino)bis[ethanol] (1:4)	NA
75150-14-0	1,4-Benzenedisulfonic acid, 2-[[4-[[4-[[1-hydroxy-6-	NA

CAS RN	DSL name	Colour Index name
	(phenylamino)-3-sulfo-2-naphthalenyl]azo]-1-naphthalenyl]azo]-6-sulfo-1-naphthalenyl]azo]-, ammonium sodium salt	
75768-93-3	2-Naphthalenesulfonic acid, 7-(benzoylamino)-4-hydroxy-3-[[4-[(4-sulfophenyl)azo]phenyl]azo]-, compd. with 2,2′,2″-nitrilotris[ethanol] (1:2)	Direct Red 81 triethanolamine salt
83221-53-8	Benzoic acid, 5-[[4-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-1-naphthalenyl]azo]-2-hydroxy-, sodium salt	NA
83221-54-9	Benzoic acid, 3-[[4-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-1-naphthalenyl]azo]-2-hydroxy-, sodium salt	NA
83221-56-1	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-(phenylazo)-, sodium salt	NA
83221-68-5	2-Naphthalenesulfonic acid, 6-[(2,4-diaminophenyl)azo]-3-[[4-[[4-[[7-[(2,4-diaminophenyl)azo]-1-hydroxy-3-sulfo-2-naphthalenyl]azo]phenyl]amino]-3-sulfophenyl]azo]-4-hydroxy-, trilithium salt	NA
83221-69-6	2-Naphthalenesulfonic acid, 6-[(2,4-diaminophenyl)azo]-3-[[4-[[4-[[7-[(2,4-diaminophenyl)azo]-1-hydroxy-3-sulfo-2-naphthalenyl]azo]phenyl]amino]-3-sulfophenyl]azo]-4-hydroxy-, lithium sodium salt	NA
83221-72-1	2,7-Naphthalenedisulfonic acid, 4-amino-3,6-bis[[4-[(2,4-diaminophenyl)azo]phenyl]azo]-5-hydroxy-, lithium sodium salt	NA
83221-73-2	Benzoic acid, 4,4′-[carbonylbis[imino(1-hydroxy-3-sulfo-6,2-naphthalenediyl)azo]]bis-, sodium salt	NA
83221-74-3	Benzoic acid, 4-[[1-hydroxy-6-[[[[5-hydroxy-6-(phenylazo)-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-3-sulfo-2-naphthalenyl]azo]-, sodium salt	NA
83232-28-4	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[3-[[4-(acetylamino)phenyl]azo]-4-hydroxy-, sodium salt	NA
83232-29-5	2-Naphthalenesulfonic acid, 3-[[4-(acetylamino)phenyl]azo]-4-hydroxy-7-[[[[5-hydroxy-6-(phenylazo)-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-, sodium salt	NA
83232-30-8	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-[(2-methylphenyl)azo]-, sodium salt	NA
83232-31-9	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-[(2-methyl-4-sulfophenyl)azo]-, sodium salt	NA
83232-32-0	2-Naphthalenesulfonic acid, 4-hydroxy-7-[[[[5-hydroxy-6-	NA

CAS RN	DSL name	Colour Index name
	[(2-methylphenyl)azo]-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-3-[(2-methyl-4-sulfophenyl)azo]-, sodium salt	
83783-94-2	2,7-Naphthalenedisulfonic acid, 3,3′-[1,2-ethenediylbis[(3-sulfo-4,1-phenylene)azo]]bis[5-amino-4-hydroxy-, lithium sodium salt, compd. with 2,2′-(methylimino)bis[ethanol]	NA
83783-95-3	2-Naphthalenesulfonic acid, 3,3′-[1,2-ethenediylbis[(3-sulfo-4,1-phenylene)azo]]bis[6-amino-4-hydroxy-, lithium sodium salt, compd. with 2,2′-(methylimino)bis[ethanol]	NA
83783-96-4	2,7-Naphthalenedisulfonic acid, 5-amino-3-[[4-[2-[4-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-2-sulfophenyl]ethenyl]-3-sulfophenyl]azo]-4-hydroxy-, lithium sodium salt, compd. with 2,2′-(methylimino)bis[ethanol]	NA
83783-99-7	Benzoic acid, 3,3′-[1,2-ethenediylbis[(3-sulfo-4,1-phenylene)azo]]bis[6-hydroxy-5-methyl-, lithium sodium salt, compd. with 2,2′-(methylimino)bis[ethanol]	NA
84878-16-0	2,7-Naphthalenedisulfonic acid, 4-amino-6-[[4-[[4-[(2,4-dihydroxyphenyl)azo]phenyl]thio]phenyl]azo]-5-hydroxy-3-[(4-nitrophenyl)azo]-, sodium salt	NA
84878-17-1	2,7-Naphthalenedisulfonic acid, 4-amino-6-[[4-[[[4-[(2,4-dihydroxyphenyl)azo]phenyl]amino]sulfonyl]phenyl]azo]-5-hydroxy-3-[(4-nitrophenyl)azo]-, potassium salt	NA
85169-18-2	Glycine, *N*-[4-[[2-[4-[[1-amino-8-hydroxy-7-(phenylazo)-3,6-disulfo-2-naphthalenyl]azo]phenyl]-1*H*-benzimidazol-5-yl]azo]-3-hydroxyphenyl]-, compd. with 2,2′-iminobis[ethanol] (1:3)	NA
85269-31-4	Benzoic acid, 3,3′-[1,2-ethenediylbis[(3-sulfo-4,1-phenylene)azo]]bis[6-hydroxy-5-methyl-, potassium salt, compd. with 2,2′,2″-nitrilotris[ethanol]	NA
93803-37-3	2,7-Naphthalenedisulfonic acid, 4-amino-5-hydroxy-3-[[4-[5-[(4-hydroxyphenyl)azo]-1*H*-benzimidazol-2-yl]phenyl]azo]-6-(phenylazo)-, disodium salt	NA
102082-94-0	2,7-Naphthalenedisulfonic acid, 4-amino-6-[[4-[[[4-[(2,4-diaminophenyl)azo]phenyl]amino]sulfonyl]phenyl]azo]-5-hydroxy-3-[(4-nitrophenyl)azo]-, lithium salt	NA
110152-63-1	Benzenesulfonic acid, 2,2′-(1,2-ethenediyl)bis[5-[(4-hydroxyphenyl)azo]-, lithium sodium salt	NA

Abbreviation: NA, not available
[a] This CAS RN is a UVCB (unknown or variable composition, complex reaction products, or biological materials).
[b] This substance was not identified under subsection 73(1) of CEPA 1999 but was included in this assessment as it was considered as a priority based on other human health concerns.

Identity of the 8 Azo Reactive Dyes in the Aromatic Azo and Benzidine-based Substance Grouping

CAS RN	DSL name	Colour Index name
17095-24-8[a]	2,7-Naphthalenedisulfonic acid, 4-amino-5-hydroxy-3,6-bis[[4-[[2-(sulfooxy)ethyl]sulfonyl]phenyl]azo]-, tetrasodium salt	Reactive Black 5
59641-46-2	2-Naphthalenesulfonic acid, 7-[[4-chloro-6-[(3-sulfophenyl)amino]-1,3,5-triazin-2-yl]amino]-4-hydroxy-3-[(4-methoxy-2-sulfophenyl)azo]-	NA
83399-85-3	1,4-Benzenedisulfonic acid, 2-[[4-[[4-[[(2,3-dichloro-6-quinoxalinyl)carbonyl]amino]-5-sulfo-1-naphthalenyl]azo]-7-sulfo-1-naphthalenyl]azo]-, lithium sodium salt	NA
83400-10-6	1,5-Naphthalenedisulfonic acid, 2-[[8-[[(2,3-dichloro-6-quinoxalinyl) carbonyl]amino]-1-hydroxy-3,6-disulfo-2-naphthalenyl]azo]-, lithium sodium salt	NA
83400-11-7	1,7-Naphthalenedisulfonic acid, 4-(benzoylamino)-6-[[5-[[(5-chloro-2,6-difluoro-4-pyrimidinyl)amino]methyl]-1-sulfo-2-naphthalenyl]azo]-5-hydroxy-, lithium sodium salt	Reactive Black 158
83400-12-8	2,7-Naphthalenedisulfonic acid, 5-(benzoylamino)-3-[[5-[[(5-chloro-2,6-difluoro-4-pyrimidinyl)amino]methyl]-1-sulfo-2-naphthalenyl]azo]-4-hydroxy-, lithium sodium salt	NA
85586-78-3	1,5-Naphthalenedisulfonic acid, 3-[[4-[[4-[(4-amino-6-chloro-1,3,5-triazin-2-yl)amino]-7-sulfo-1-naphthalenyl]azo]-7-sulfo-1-naphthalenyl]azo]-, potassium sodium salt	NA
108624-00-6	2,7-Naphthalenedisulfonic acid, 4-amino-6-[[5-[(5-chloro-2,6-difluoro-4-pyrimidinyl)amino]-2-sulfophenyl]azo]-5-hydroxy-3-[[4-[[2-(sulfooxy)ethyl]sulfonyl]phenyl]azo]-, lithium sodium salt	Reactive Blue 225

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

Azo direct dyes and azo reactive dyes are not expected to occur naturally in the environment. No manufacture of any substance above the 100 kg/year reporting threshold has been reported in Canada in response to any recent surveys under section

71 of CEPA 1999. Seven substances (six azo direct dyes and one azo reactive dye) have been reported as having an import quantity above the 100 kg/year survey reporting threshold. Some of these substances were also among those identified as being used in products available to consumers in the Canadian marketplace. Azo direct dyes are generally used for colouring of textile, leather and paper. Azo reactive dyes are used primarily in the textiles industry for dyeing cellulosic fibres such as cotton and rayon.

Environment

All azo direct dyes and azo reactive dyes are soluble in water, with solubilities generally well above 1 g/L. When considering potential releases to water, sediment and soil and the physical and chemical properties of these substances, it is expected that the azo direct and azo reactive dyes may remain in the water column for relatively long periods of time due to their hydrophilicity, but will ultimately partition via electrostatic interactions to suspended solids, sediments or soil particles. Available experimental and modelled data regarding the abiotic and biotic degradation of the azo direct and azo reactive dyes indicate that these substances may persist in water, sediment and soil. In anaerobic environments (e.g., anoxic layers of sediments), there is the potential for these substances to degrade to aromatic amines as a result of cleavage of the azo bond under anaerobic or reducing conditions.

Although there are limited experimental data available, information on the log octanol–water partition coefficients and fish bioconcentration factors indicates that these substances are not likely to bioconcentrate or bioaccumulate in aquatic organisms.

There is a wide range of acute toxicity data for azo direct dyes (median lethal concentrations [LC_{50}] ranging from 75 to ≥ 1000 mg/L), the most sensitive being a rainbow trout 48-hour LC_{50} of 75 mg/L. Azo reactive dyes were found to elicit effects in aquatic organisms at low concentrations. The aquatic invertebrate *Daphnia magna* was found to be more sensitive than the various fish species tested. The differences were even more pronounced when the length of exposure (up to 21 days) was increased. Daphnid reproduction was found to be the most sensitive endpoint, with a 21-day no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC) of 1.25 and 2.5 mg/L, respectively. Soil and sediment toxicity data are not available for these substances.

Aquatic exposure analyses were conducted for scenarios representing potential major environmental releases due to industrial activities involving azo direct and azo reactive dyes. The likelihood of the PECs exceeding the predicted no-effect concentrations (PNECs) for azo direct dyes and azo reactive dyes was found to be low.

Considering all available lines of evidence presented in this screening assessment, there is low risk of harm to organisms and the broader integrity of the environment from azo direct and azo reactive dyes evaluated in this assessment. It is concluded that these 61 azo direct dyes and eight azo reactive dyes do not meet the criteria under paragraph 64*(a)* or 64*(b)* of CEPA 1999, as they are not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

Human Health

Azo Direct Dyes

For the general population of Canada, environmental media are not considered to be a significant source of exposure to the azo direct dyes; therefore risk to human health from this source is considered to be low. Among the 61 azo direct dyes, 18 have been identified to be used in products available to consumers in the Canadian marketplace. These dyes are Direct Black 56, Direct Blue 71, Direct Green 28, Direct Orange 26, Direct Orange 39, Direct Red 31, Direct Red 81 triethanolamine salt, Direct Violet 51, Direct Yellow 11, Direct Yellow 11 lithium salt, Direct Yellow 12, Direct Yellow 28, Direct Yellow 34, Direct Yellow 50, CAS RN 28706-21-0, CAS RN 71033-21-1, CAS RN 83221-56-1 and CAS RN 84878-17-1.

Margins between the exposure estimates for 14 of the azo direct dyes (Direct Black 56, Direct Green 28, Direct Orange 26, Direct Orange 39, Direct Red 81 triethanolamine salt, Direct Violet 51, Direct Yellow 28, Direct Red 31, Direct Yellow 12, Direct Yellow 50, CAS RN 28706-21-0, CAS RN 71033-21-1, CAS RN 83221-56-1 and CAS RN 84878-17-1) used as dyes in textiles and the critical health effects levels were considered adequate to address uncertainties in the exposure and health effects databases.

Exposure to nine of the azo direct dyes (Direct Black 56, Direct Red 31, Direct Red 81 triethanolamine salt, Direct Yellow 12, Direct Yellow 50, CAS RN 28706-21-0, CAS RN 71033-21-1, CAS RN 83221-56-1 and CAS RN 84878-17-1) used as dyes in leather products is considered to be short term and intermittent. Therefore, the margins of exposure derived for these dyes used in textiles are considered to be protective for individuals using leather products.

Seven of the azo direct dyes (Direct Blue 71, Direct Red 31, Direct Yellow 11, Direct Yellow 11 lithium salt, Direct Yellow 12, Direct Yellow 34 and Direct Yellow 50) were identified to be used as dyes in paper products. It is recognised that young children may be occasionnaly exposed to these dyes from incidental ingestion of paper products. However, available data do not indicate acute toxicity to be a health concern for the azo direct dyes, therefore, risk to young children from exposure to these dyes in paper products is considered to be low.

Exposure to Direct Blue 71 and Direct Yellow 11 lithium salt used as dyes in food packaging materials is not expected to be significant, therefore risk to human health is considered to be low for this use.

For the remaining azo direct dyes, available information did not identify sources of current exposure for the general population of Canada, therefore risk to human health is not expected for these substances.

Some of the azo direct dyes in this assessment have effects of concern based on potential carcinogenicity.

Based on the information presented in this screening assessment, it is concluded that the above azo direct dyes 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.

Azo Reactive Dyes

For the general population of Canada, environmental media are not considered to be a significant source of exposure to the azo reactive dyes; therefore risk to human health from this source is considered to be low. Use of Reactive Black 5, Reactive Black 158 and Reactive Blue 225 as covalently bound dyes in textiles is not expected to be a significant source of exposure, therefore the risk to human health is considered to be low for these substances. For the remaining five azo reactive dyes, available information did not identify sources of current exposure for the general population of Canada, therefore risk to human health is not expected for these substances.

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

Overall Conclusion

It is concluded that the 61 azo direct dyes and 8 azo reactive dyes evaluated in this assessment do not meet any of the criteria set out in section 64 of CEPA 1999.

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1. Introduction

Pursuant to 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, diversity within the substance group has been taken into account through the establishment of subgroups. Subgrouping based on structural similarities, physical and chemical properties, and common functional uses and applications accounts for variability within this substance grouping and allows for subgroup-specific approaches in the conduct of screening assessments. This Screening Assessment considers substances that belong to the Azo Direct Dyes and Azo Reactive Dyes 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, 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).

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 based on a weight of evidence approach and using 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 August 2013. Empirical data from key studies as well as some results from models were used to reach conclusions. When available and relevant, information presented in assessments from other jurisdictions was considered.

The Screening Assessment does not represent an exhaustive 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/or consultation. Comments on the technical portions relevant to the environment were received from Dr. Harold Freeman (North Carolina State University, USA) and Dr. Gisela Umbuzeiro (University of Campinas, Brazil). Comments on the technical portions relevant to human health were received from Dr. Harold Freeman (North Carolina State University, USA), Dr. David Josephy (University of Guelph, Canada), Dr. Michael Bird (University of Ottawa, Canada) and Dr. Kannan Krishnan (Université de Montréal, Canada). Additionally, the 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.

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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 1999 or other Acts.

2. Identity of Substances

This Screening Assessment focuses on 69 substances that belong to two subgroups that are part of the Aromatic Azo and Benzidine-based Substance Grouping. These subgroups are based on structural similarity and similar applications: Azo Direct Dyes and Azo Reactive Dyes.

The Azo Direct Dyes subgroup includes three subsets based on the number of azo functional groups: monoazo (one azo functional group), disazo (two azo functional groups) and polyazo (three or four azo functional groups, known as trisazo and tetrakisazo, respectively). For ecological purposes, monoazo, disazo and polyazo subsets from the Azo Direct Dyes subgroup are discussed independently when sufficient data are available, as there is some variation in the molecular weights, certain physical and chemical properties and ecotoxicity of substances in the three subsets. The Azo Reactive Dyes are addressed together due to similarities in their physical and chemical properties and usage.

The subsets and identities of the individual Azo Direct Dyes in each subset are presented in Tables 2-1 to 2-6. The identities of the individual Azo Reactive Dyes are shown in Tables 2-7 and 2-8. When available, a Colour Index (C.I.) name or recognized common name is used to identify the substance. If these terms are unavailable, the Chemical Abstracts Service Registry Number (CAS RN) is used as the primary identifier. A list of additional chemical names (e.g., trade names) is available from the National Chemical Inventories (NCI 2012).

Table 2-1. Description and representative chemical structure of the five Monoazo Direct Dyes

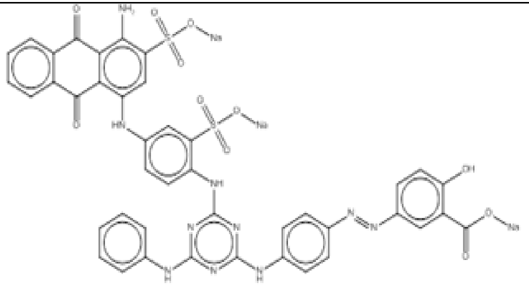
Subset	Representative chemical structure of the subset	Group description with critical functional groups	Molar weight range (g/mol)
Azo Direct Dyes (Monoazo; *n* = 5)	 $C_{42}H_{27}N_{10}Na_3O_{11}S_2$ CAS RN 6471-09-6 (Direct Green 28)	Seven rings, monoazos with a sulfonate group	592–980

Table 2-2. Identity of the five Monoazo Direct Dyes

CAS RN	DSL name	C.I. generic name	Molar weight (g/mol)
1325-37-7[a]	C.I. Direct Yellow 11	Direct Yellow 11	624
6471-09-6	Benzoic acid, 5-[[4-[[4-[[4-[(4-amino-9,10-dihydro-9,10-dioxo-3-sulfo-1-anthracenyl)amino]-2-sulfophenyl]amino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]phenyl]azo]-2-hydroxy-, trisodium salt	Direct Green 28	980
10114-47-3	7-Benzothiazolesulfonic acid, 2,2′-(azodi-4,1-phenylene)bis[6-methyl-, disodium salt	Direct Yellow 28	680
65150-80-3[a]	C.I. Direct Yellow 11, lithium salt	Direct Yellow 11 lithium salt	592
71033-21-1[a]	Benzothiazolesulfonic acid, 2,2′-(azodi-4,1-phenylene)bis[6-methyl-, disodium salt	NA	682

[a] This CAS RN is a UVCB (unknown or variable composition, complex reaction products, or biological materials).

Table 2-3. Description and representative chemical structure of the 37 Disazo Direct Dyes

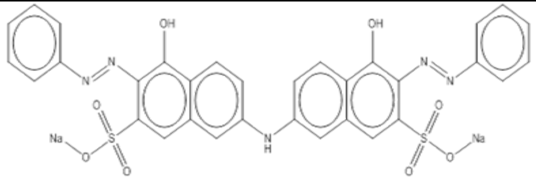
Subset	Representative chemical structure of the subset	Group description with critical functional groups	Molar weight range (g/mol)
Azo Direct Dyes (Disazo; *n* = 37)	 $C_{32}H_{21}N_5Na_2O_8S_2$ CAS RN 5001-72-9 (Direct Red 31)	Dyes with a urea bridge HN-CO-NH bridging two phenyl amines are found in this class. Co-planar molecule. Similar to the basic structure of Direct Orange 26.	584–1397

Table 2-4. Identity of the 37 Disazo Direct Dyes

CAS RN	DSL name	C.I. generic name	Molar weight (g/mol)
2829-42-7	Benzoic acid, 3,3′-[carbonylbis(imino-4,1-phenyleneazo)]bis[6-hydroxy-, disodium salt	Direct Yellow 26	584
2870-32-8	Benzenesulfonic acid, 2,2′-(1,2-ethenediyl)bis[5-[(4-ethoxyphenyl)azo]-, disodium salt	Direct Yellow 12	680
3214-47-9	1,5-Naphthalenedisulfonic acid, 3,3′-[carbonylbis[imino(2-methyl-4,1-phenylene)azo]]bis-, tetrasodium salt	Direct Yellow 50	956
3626-36-6	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-(phenylazo)-, disodium salt	Direct Orange 26	938
3687-80-7	1-Naphthalenesulfonic acid, 4-[[1-hydroxy-6-[[[[5-hydroxy-6-[(2-methoxyphenyl)azo]-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-3-sulfo-2-naphthalenyl]azo]-, trisodium salt	Direct Red 26	938
5001-72-9	2-Naphthalenesulfonic acid, 7,7′-iminobis[4-hydroxy-3-(phenylazo)-, disodium salt	Direct Red 31	713
5489-77-0	2-Naphthalenesulfonic acid, 3-[[4-[(2,4-dimethyl-6-sulfophenyl)azo]-2-methoxy-5-methylphenyl]azo]-4-hydroxy-7-(phenylamino)-, disodium salt	Direct Violet 51	719
6420-33-3	1,5-Naphthalenedisulfonic acid, 3,3′-[carbonylbis[imino(5-methoxy-2-methyl-4,1-phenylene)azo]]bis-, tetrasodium salt	Direct Yellow 34	1016
6420-41-3	2-Naphthalenesulfonic acid, 4-hydroxy-7-[[[[5-hydroxy-6-(phenylazo)-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-3-[(6-sulfo-2-naphthalenyl)azo]-, trisodium salt	Direct Red 4	908
6420-43-5	2-Naphthalenesulfonic acid, 4-hydroxy-7-[[[[5-hydroxy-6-[(2-methylphenyl)azo]-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-3-[(2-methyl-4-sulfophenyl)azo]-, trisodium salt	Direct Red 62	886
12217-64-0	1,3-Naphthalenedisulfonic acid, 7,7′-[carbonylbis[imino(5-methoxy-2-methyl-	Direct Orange 72	1016

CAS RN	DSL name	C.I. generic name	Molar weight (g/mol)
	4,1-phenylene)azo]]bis-, tetrasodium salt		
28706-21-0	1,3-Naphthalenedisulfonic acid, 7,7′-[iminobis[carbonyl(2-methyl-4,1-phenylene)azo]]bis-, tetrasodium salt	NA	956
38801-08-0	Benzoic acid, 4,4′-[carbonylbis[imino(1-hydroxy-3-sulfo-6,2-naphthalenediyl)azo]]bis-, compd. with 2,2′,2″-nitrilotris[ethanol] (1:4)	NA	1397
53523-90-3	Benzoic acid, 3,3′-[1,2-ethenediylbis[(3-sulfo-4,1-phenylene)azo]]bis[6-hydroxy-5-methyl-, tetralithium salt	NA	843
72139-21-0	Benzoic acid, 3,3′-[(1,4-dioxo-2-butene-1,4-diyl)bis(imino-4,1-phenyleneazo)]bis[6-hydroxy-, disodium salt	NA	638
72152-50-2	Benzoic acid, 2-[[6-[[4-[[6-(benzoylamino)-1-hydroxy-3-sulfo-2-naphthalenyl]azo]-3-methylbenzoyl]amino]-1-hydroxy-3-sulfo-2-naphthalenyl]azo]-, trisodium salt	NA	940
72245-49-9	Benzoic acid, 4-[[1-hydroxy-6-[[[[5-hydroxy-6-[(2-methyl-4-sulfophenyl)azo]-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-3-sulfo-2-naphthalenyl]azo]-, sodium salt	NA	938
72749-87-2	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-[(2-methylphenyl)azo]-, disodium salt	NA	784
72749-88-3	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-[(2-methoxyphenyl)azo]-, disodium salt	NA	816
72869-93-3	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-[(6-sulfo-2-naphthalenyl)azo]-, compd. with 2,2′-(methylimino)bis[ethanol] (1:4)	NA	724
75768-93-3	2-Naphthalenesulfonic acid, 7-(benzoylamino)-4-hydroxy-3-[[4-[(4-sulfophenyl)azo]phenyl]azo]-, compd. with 2,2′,2″-nitrilotris[ethanol] (1:2)	Direct Red 81 triethanolamine salt	813
83221-53-8	Benzoic acid, 5-[[4-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-1-naphthalenyl]azo]-2-hydroxy-, sodium salt	NA	601
83221-54-9	Benzoic acid, 3-[[4-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-1-	NA	601

CAS RN	DSL name	C.I. generic name	Molar weight (g/mol)
	naphthalenyl]azo]-2-hydroxy-, sodium salt		
83221-56-1	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-(phenylazo)-, sodium salt	NA	736
83221-73-2	Benzoic acid, 4,4′-[carbonylbis[imino(1-hydroxy-3-sulfo-6,2-naphthalenediyl)azo]]bis-, sodium salt	NA	888
83221-74-3	Benzoic acid, 4-[[1-hydroxy-6-[[[[5-hydroxy-6-(phenylazo)-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-3-sulfo-2-naphthalenyl]azo]-, sodium salt	NA	822
83232-28-4	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[3-[[4-(acetylamino)phenyl]azo]-4-hydroxy-, sodium salt	NA	850
83232-29-5	2-Naphthalenesulfonic acid, 3-[[4-(acetylamino)phenyl]azo]-4-hydroxy-7-[[[[5-hydroxy-6-(phenylazo)-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-, sodium salt	NA	813
83232-30-8	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-[(2-methylphenyl)azo]-, sodium salt	NA	NA
83232-31-9	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-[(2-methyl-4-sulfophenyl)azo]-, sodium salt	NA	924
83232-32-0	2-Naphthalenesulfonic acid, 4-hydroxy-7-[[[[5-hydroxy-6-[(2-methylphenyl)azo]-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-3-[(2-methyl-4-sulfophenyl)azo]-, sodium salt	NA	886
83783-94-2	2,7-Naphthalenedisulfonic acid, 3,3′-[1,2-ethenediylbis[(3-sulfo-4,1-phenylene)azo]]bis[5-amino-4-hydroxy-, lithium sodium salt, compd. with 2,2′-(methylimino)bis[ethanol]	NA	1249
83783-95-3	2-Naphthalenesulfonic acid, 3,3′-[1,2-ethenediylbis[(3-sulfo-4,1-phenylene)azo]]bis[6-amino-4-hydroxy-, lithium sodium salt, compd. with 2,2′-(methylimino)bis[ethanol]	NA	1061
83783-96-4	2,7-Naphthalenedisulfonic acid, 5-amino-	NA	1147

CAS RN	**DSL name**	**C.I. generic name**	**Molar weight (g/mol)**
	3-[[4-[2-[4-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-2-sulfophenyl]ethenyl]-3-sulfophenyl]azo]-4-hydroxy-, lithium sodium salt, compd. with 2,2′-(methylimino)bis[ethanol]		
83783-99-7	Benzoic acid, 3,3′-[1,2-ethenediylbis[(3-sulfo-4,1-phenylene)azo]]bis[6-hydroxy-5-methyl-, lithium sodium salt, compd. with 2,2′-(methylimino)bis[ethanol]	NA	843
85269-31-4	Benzoic acid, 3,3′-[1,2-ethenediylbis[(3-sulfo-4,1-phenylene)azo]]bis[6-hydroxy-5-methyl-, potassium salt, compd. with 2,2′,2″-nitrilotris[ethanol]	NA	812
110152-63-1	Benzenesulfonic acid, 2,2′-(1,2-ethenediyl)bis[5-[(4-hydroxyphenyl)azo]-, lithium sodium salt	NA	802

Table 2-5. Description and representative chemical structure of the 19 Polyazo Direct Dyes

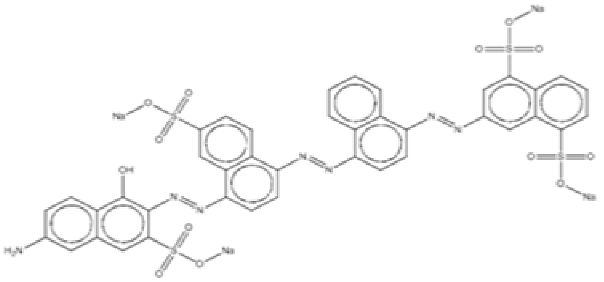
Subset	**Representative chemical structure of the subset**	**Group description with critical functional groups**	**Molar weight range (g/mol)**
Azo Direct Dyes (Polyazo; *n* = 19)	 $C_{40}H_{23}N_7Na_4O_{13}S_4$ CAS RN 4399-55-7 (Direct Blue 71)	Trisazo, non-benzidine, 3 sets of naphthalene rings, 7–8 rings in total, all – water-soluble salts	635–1050

Table 2-6. Identity of the 19 Polyazo Direct Dyes

CAS RN	DSL name	C.I. generic name	Molar weight (g/mol)
1325-54-8[a]	Benzenesulfonic acid, 2,2′-(1,2-ethenediyl)bis-5-nitro-, disodium salt, reaction products with 4-[(4-aminophenyl)azo]benzenesulfonic acid, sodium salts	Direct Orange 39	990
4399-55-7	1,5-Naphthalenedisulfonic acid, 3-[[4-[[4-[(6-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-6-sulfo-1-naphthalenyl]azo]-1-naphthalenyl]azo]-, tetrasodium salt	Direct Blue 71	1029
6406-87-7	2-Naphthalenesulfonic acid, 5-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-8-[[4-(phenylazo)-7-sulfo-1-naphthalenyl]azo]-, trisodium salt	NA	877
6476-10-4	2-Naphthalenesulfonic acid, 8-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-5-[[4-(phenylazo)-6-sulfo-1-naphthalenyl]azo]-, trisodium salt	NA	877
10134-33-5	2-Naphthalenesulfonic acid, 8-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-5-[[4-(phenylazo)-7-sulfo-1-naphthalenyl]azo]-, trisodium salt	Direct Black 56	877
10482-42-5	2-Naphthalenesulfonic acid, 5-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-8-[[4-(phenylazo)-6-sulfo-1-naphthalenyl]azo]-, trisodium salt	NA	877
32829-81-5	Benzenesulfonic acid, 2,2′-(1,2-ethenediyl)bis[5-[[4-[(4-sulfophenyl)azo]phenyl]azo]-, tetrasodium salt	NA	1004
71767-19-6	2-Naphthalenesulfonic acid, 5-[[6-amino-1-hydroxy-3-sulfo-5-[(3-sulfophenyl)azo]-2-naphthalenyl]azo]-6-methoxy-8-[[7-sulfo-4-[(3-sulfophenyl)azo]-1-naphthalenyl]azo]-, pentasodium salt	NA	1050
71873-49-9	Benzoic acid, 4,4′-[1,2-ethenediylbis[(3-sulfo-4,1-phenylene)-ONN-azoxy-4,1-phenyleneazo]]bis-, tetrasodium salt	NA	964
72245-56-8	2,7-Naphthalenedisulfonic acid, 4-amino-3-[[4-[[[4-[(2,4-diaminophenyl)azo]phenyl]amino] carbonyl]phenyl]azo]-5-hydroxy-6-(phenylazo)-, sodium salt	NA	824
75150-14-0	1,4-Benzenedisulfonic acid, 2-[[4-[[4-[[1-hydroxy-6-(phenylamino)-3-sulfo-2-naphthalenyl]azo]-1-naphthalenyl]azo]-6-sulfo-1-naphthalenyl]azo]-, ammonium sodium salt	NA	1046
83221-68-5	2-Naphthalenesulfonic acid, 6-[(2,4-diaminophenyl)azo]-3-[[4-[[4-[[7-[(2,4-diaminophenyl)azo]-1-hydroxy-3-sulfo-2-naphthalenyl]azo]phenyl]amino]-3-sulfophenyl]azo]-4-hydroxy-, trilithium salt	NA	635
83221-	2-Naphthalenesulfonic acid, 6-[(2,4-	NA	1050

CAS RN	DSL name	C.I. generic name	Molar weight (g/mol)
69-6	diaminophenyl)azo]-3-[[4-[[4-[[7-[(2,4-diaminophenyl)azo]-1-hydroxy-3-sulfo-2-naphthalenyl]azo]phenyl]amino]-3-sulfophenyl]azo]-4-hydroxy-, lithium sodium salt		
83221-72-1	2,7-Naphthalenedisulfonic acid, 4-amino-3,6-bis[[4-[(2,4-diaminophenyl)azo]phenyl]azo]-5-hydroxy-, lithium sodium salt	NA	823
84878-16-0	2,7-Naphthalenedisulfonic acid, 4-amino-6-[[4-[[4-[(2,4-dihydroxyphenyl)azo]phenyl]thio]phenyl]azo]-5-hydroxy-3-[(4-nitrophenyl)azo]-, sodium salt	NA	860
84878-17-1	2,7-Naphthalenedisulfonic acid, 4-amino-6-[[4-[[[4-[(2,4-dihydroxyphenyl)azo]phenyl]amino]sulfonyl]phenyl]azo]-5-hydroxy-3-[(4-nitrophenyl)azo]-, potassium salt	NA	939
85169-18-2	Glycine, *N*-[4-[[2-[4-[[1-amino-8-hydroxy-7-(phenylazo)-3,6-disulfo-2-naphthalenyl]azo]phenyl]-1*H*-benzimidazol-5-yl]azo]-3-hydroxyphenyl]-, compd. with 2,2′-iminobis[ethanol] (1:3)	NA	801
93803-37-3	2,7-Naphthalenedisulfonic acid, 4-amino-5-hydroxy-3-[[4-[5-[(4-hydroxyphenyl)azo]-1*H*-benzimidazol-2-yl]phenyl]azo]-6-(phenylazo)-, disodium salt	NA	807
102082-94-0	2,7-Naphthalenedisulfonic acid, 4-amino-6-[[4-[[[4-[(2,4-diaminophenyl)azo]phenyl]amino]sulfonyl]phenyl]azo]-5-hydroxy-3-[(4-nitrophenyl)azo]-, lithium salt	NA	873

[a] This CAS RN is a UVCB (unknown or variable composition, complex reaction products, or biological materials).

Table 2-7. Description and representative chemical structure of the eight Azo Reactive Dyes

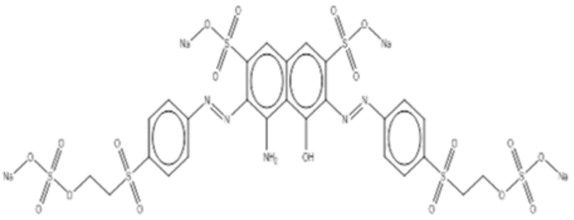
Subgroup	Representative chemical structure of the subgroup	Group description with critical functional groups	Molar weight range (g/mol)
Azo Reactive Dyes (*n* = 8)	 $C_{26}H_{21}N_5Na_4O_{19}S_6$ CAS RN 17095-24-8 (Reactive Black 5)	Reactive, diazo, non-benzidine, 1 set of naphthalene rings, 4–6 rings in total, all – water-soluble salts	738–1015

Table 2-8. Identity of the eight Azo Reactive Dyes

CAS RN	DSL name	C.I. generic name	Molar weight (g/mol)
17095-24-8	2,7-Naphthalenedisulfonic acid, 4-amino-5-hydroxy-3,6-bis[[4-[[2-(sulfooxy)ethyl]sulfonyl]phenyl]azo]-, tetrasodium salt	Reactive Black 5	991
59641-46-2	2-Naphthalenesulfonic acid, 7-[[4-chloro-6-[(3-sulfophenyl)amino]-1,3,5-triazin-2-yl]amino]-4-hydroxy-3-[(4-methoxy-2-sulfophenyl)azo]-	NA	738
83399-85-3	1,4-Benzenedisulfonic acid, 2-[[4-[[4-[[(2,3-dichloro-6-quinoxalinyl)carbonyl]amino]-5-sulfo-1-naphthalenyl]azo]-7-sulfo-1-naphthalenyl]azo]-, lithium sodium salt	NA	1002
83400-10-6	1,5-Naphthalenedisulfonic acid, 2-[[8-[[(2,3-dichloro-6-quinoxalinyl) carbonyl]amino]-1-hydroxy-3,6-disulfo-2-naphthalenyl]azo]-, lithium sodium salt	NA	914
83400-11-7	1,7-Naphthalenedisulfonic acid, 4-(benzoylamino)-6-[[5-[[(5-chloro-2,6-difluoro-4-pyrimidinyl)amino]methyl]-1-sulfo-2-naphthalenyl]azo]-5-hydroxy-, lithium sodium salt	Reactive Black 158	885
83400-12-8	2,7-Naphthalenedisulfonic acid, 5-(benzoylamino)-3-[[5-[[(5-chloro-2,6-difluoro-4-pyrimidinyl)amino]methyl]-1-sulfo-2-naphthalenyl]azo]-4-hydroxy-, lithium sodium salt	NA	885
85586-78-3	1,5-Naphthalenedisulfonic acid, 3-[[4-[[4-[(4-amino-6-chloro-1,3,5-triazin-2-yl)amino]-7-sulfo-1-naphthalenyl]azo]-7-sulfo-1-naphthalenyl]azo]-, potassium sodium salt	NA	964
108624-00-6	2,7-Naphthalenedisulfonic acid, 4-amino-6-[[5-[(5-chloro-2,6-difluoro-4-pyrimidinyl)amino]-2-sulfophenyl]azo]-5-hydroxy-3-[[4-[[2-(sulfooxy)ethyl]sulfonyl]phenyl]azo]-, lithium sodium salt	Reactive Blue 225	1015

More detailed information regarding the identity of these substances can be found in Appendix A (Tables A1–A4).

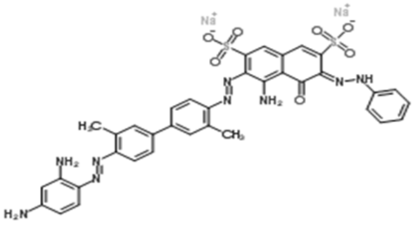
2.1 Selection of Analogues and Use of (Q)SAR Models

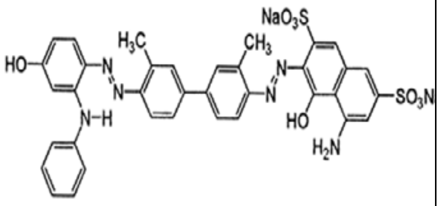
Guidance on the use of read-across approaches has been prepared by various organizations, such as the Organisation for Economic Co-operation and Development (OECD 2014). It has been applied in various regulatory programs, including the European Union's (EU) Existing Substances Programme. The general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

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

The two analogues used to inform this assessment are presented in Table 2-9, along with an indication of the potential read-across data available for different parameters.

Table 2-9. Identification of two analogues for Polyazo Direct Dyes and parameters to be used to inform the environmental fate and potential to cause ecological harm

CAS RN	Chemical structure, C.I. name and formula	Molar weight (g/mol)	Parameters to be used in the report	Description
37372-50-2	 Direct Black 154 $C_{36}H_{29}N_9Na_2O_7S_2$	809	BCF	Bioconcentration factor

CAS RN	Chemical structure, C.I. name and formula	Molar weight (g/mol)	Parameters to be used in the report	Description
6459-98-9	 Direct Black 30 $C_{36}H_{28}N_9Na_2O_8S_2$	782	LC_{50}	As there were no empirical data for the Polyazo Direct Dyes subset

Abbreviations: BCF, bioconcentration factor; LC_{50}, the concentration of a substance that is estimated to be lethal to 50% of the test organisms

3. Physical and Chemical Properties

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

Several physical and chemical properties of Azo Direct and Azo Reactive Dyes—namely, melting point, water solubility, size, log octanol–water partition coefficient (log K_{ow}) and acid dissociation constant (pK_a)—are important in terms of ecological and human health assessment. A summary of the experimental physical and chemical properties relevant to the environmental fate and ecotoxicity of the Monoazo, Disazo and Polyazo Direct Dyes (and the two Polyazo Direct Dye analogues) is presented in Tables 3-1, 3-2 and 3-3, respectively. A summary of the experimental physical and chemical properties relevant to the environmental fate and ecotoxicity of the Azo Reactive Dyes is presented in Table 3-4. 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 subset or subgroup. Detailed substance-specific information on these properties can be found in Tables A5 and A6 in Appendix A of this report.

The Azo Direct Dyes in this assessment have a wide range of molecular weights (584–1397 g/mol), while those of Azo Reactive Dyes are somewhat more confined (738–1015 g/mol). Most Azo Direct and Azo Reactive Dyes are complex anionic molecules that tend to dissociate at environmentally relevant pH levels and are highly soluble in water (solubilities ranging from 0.02 to 190 g/L) due to the presence of solubilizing functional groups (Hunger 2003; Tables 3-1 to 3-4). In general, molecular interactions between direct dyes and the application medium are via relatively weak van der Waals interactions, leading to poor wet fastness. In contrast, reactive dyes can chemically react with functional groups on the application medium to form a covalent bond between dye molecule and textile. As a result, reactive dyes have high wet fastness.

Azo Direct Dyes and Azo Reactive Dyes contain functional groups, such as sulfonic and carboxylic acids, that increase their solubility. Given their hydrophilicity and ionic character, as demonstrated by low pK_a values (an indicator of acid dissociation), Azo Direct and Azo Reactive Dyes are believed to have very low (even negative) experimental log K_{ow} values.

Table 3-1. A summary of experimental physical and chemical properties for the Monoazo Direct Dyes subset

Property	Value(s) or range (number of data points)	Pivotal value(s) for this risk assessment
Molecular weight (g/mol)[a]	592–980 (*n* = 5)	592–980 (range was used)

Property	**Value(s) or range (number of data points)**	**Pivotal value(s) for this risk assessment**
Melting point (°C)	< −12, 390 (*n* = 2)	< −12 –390 (range was used)
Water solubility (g/L)	0.02–190 (*n* = 3)	0.02–190 (range was used)
Log K_{ow} (dimensionless)	NA	NA
D_{min} (nm)[a]	0.89 (n = 1)	0.89
D_{max} (nm)[a]	1.27 (n = 1)	1.27
pK_a (dimensionless)	$pK_{a1} = 0.2$ $pK_{a2} = -0.4$	$pK_{a1} = 0.2$ $pK_{a2} = -0.4$

Abbreviations: D_{max}, maximum cross-sectional diameter; D_{min}, minimum cross-sectional diameter; K_{ow}, octanol–water partition coefficient; pK_a, acid dissociation constant

[a] Molecular weight and cross-sectional diameter ranges are shown here for the five Monoazo Direct Dyes included in this assessment only.

Table 3-2. A summary of experimental physical and chemical properties for the Disazo Direct Dyes subset

Property	**Value(s) or range (number of data points)**	**Pivotal value(s) for this risk assessment**
Molecular weight (g/mol)[a]	582–1397 (*n* = 37)	582–1397 (range was used)
Melting point (°C)	174 (*n* = 1)	174
Water solubility (g/L)	20, 30 (*n* = 2)	20– 30 (range was used)
Log K_{ow} (dimensionless)	NA	NA
D_{min} (nm)[a]	0.94–1.02 (*n* = 4)	0.94–1.02 (range was used)
D_{max} (nm)[a]	1.38–1.85 (*n* = 4)	1.38–1.85 (range was used)
pK_a (dimensionless)	$pK_{a1} = -1.6$ $pK_{a2} = -0.05$	$pK_{a1} = -1.6$ $pK_{a2} = -0.05$

Abbreviations: D_{max}, maximum cross-sectional diameter; D_{min}, minimum cross-sectional diameter; K_{ow}, octanol–water partition coefficient; pK_a, acid dissociation constant

[a] Molecular weight and cross-sectional diameter ranges are shown here for the 37 Disazo Direct Dyes included in this assessment only.

Table 3-3. A summary of experimental physical and chemical properties for the Polyazo Direct Dyes subset

Property	**Value(s) or range (number of data points)**	**Pivotal value(s) for this risk assessment**
Molecular weight (g/mol)[a]	635–1050 (*n* = 19)	635–1050 (range was used)
Melting point (°C)	175 (*n* = 1)	175
Water solubility (g/L)	20–100 (*n* = 4)	20–100 (range was used)
Log K_{ow} (dimensionless)	NA	NA
D_{min} (nm)[a]	1.06–1.19 (*n* = 2)	1.06–1.19 (range was used)

Property	Value(s) or range (number of data points)	Pivotal value(s) for this risk assessment
D_{max} (nm)[a]	1.64–1.77 (*n* = 2)	1.64–1.77 (range was used)
pK_a (dimensionless)	$pK_{a1} = 1.2$ $pK_{a2} = −0.5$	$pK_{a1} = 1.2$ $pK_{a2} = −0.5$

Abbreviations: D_{max}, maximum cross-sectional diameter; D_{min}, minimum cross-sectional diameter; K_{ow}, octanol–water partition coefficient; pK_a, acid dissociation constant

[a] Molecular weight and cross-sectional diameter ranges are shown here for the 19 Polyazo Direct Dyes included in this assessment only (i.e., analogue data are not included).

Table 3-4. A summary of experimental physical and chemical properties for the Azo Reactive Dyes

Property	Value(s) or range (number of data points)	Pivotal value(s) for this risk assessment
Molecular weight (g/mol)[a]	738–1015 (*n* = 8)	738–1015 (range was used)
Melting point (°C)	180–300 (*n* = 4)	180–300 (range was used)
Water solubility (g/L)	40– 60 (*n* = 4)	40–60 (range was used)
Log K_{ow} (dimensionless)	NA	NA
D_{min} (nm)[a]	1.00, 1.10 (*n* = 2)	1.00–1.10 (range was used)
D_{max} (nm)[a]	1.38, 1.56 (*n* = 2)	1.38–1.56 (range was used)
pK_a (dimensionless)	$pK_{a1} = −1.2$ $pK_{a2} = −0.5$	$pK_{a1} = −1.2$ $pK_{a2} = −0.5$

Abbreviations: D_{max}, maximum cross-sectional diameter; D_{min}, minimum cross-sectional diameter; K_{ow}, octanol–water partition coefficient; pK_a, acid dissociation constant

[a] Molecular weight and cross-sectional diameter ranges are shown here for the eight Azo Reactive Dyes included in this assessment only.

Azo Reactive Dyes tend to have slightly higher molecular weights and lower water solubilities than Azo Direct Dyes, but no apparent differences in physical and chemical properties. Cross-sectional diameters and lower water solubility, and are discussed further in Section 5.5.

Similar to other azo colourants, Azo Direct and Azo Reactive Dyes may undergo tautomerization between the azo and hydrazone forms. This tautomerization is well known for the azo dyes, where a hydroxyl group and the azo bond are present in the *ortho or para* position. The tautomeric forms may differ in colour, performance properties, toxicological profile and tinctorial strength (Environment Canada and Health Canada 2013). However, the degree to which this affects the fate and behaviour of these substances in the environment or their toxicological properties and processes is still not fully understood.

4. Sources and Uses

4.1 Sources

All of the Azo Direct Dyes and Azo Reactive Dyes are anthropogenically produced and are not expected to occur naturally in the environment.

In recent years (2005 to present), all 69 substances identified in this Screening Assessment have been included in surveys issued pursuant to section 71 of CEPA 1999. These surveys aimed to collect information on manufacturing and importing activities in Canada with a reporting threshold of 100 kg/year.

No manufacturing activity in Canada was reported for these substances, but six Azo Direct Dyes and one Azo Reactive Dye were reported as being imported into Canada above the 100 kg/year threshold (Canada 2011). Import quantities for the seven substances are provided in Table 4-1.

Table 4-1. Annual import quantity ranges identified in section 71 survey for the year 2010 (Canada 2011)

Subgroup	Name	Annual import quantity ranges (kg)
Azo Direct Dyes	Direct Yellow 11	1 000 – 10 000
Azo Direct Dyes	Direct Yellow 11 lithium salt	10 000 – 100 000
Azo Direct Dyes	Direct Orange 26	100 – 1 000
Azo Direct Dyes	Direct Yellow 34	100 – 1 000
Azo Direct Dyes	Direct Orange 39	100 – 1 000
Azo Direct Dyes	Direct Blue 71	1 000 – 10 000
Azo Reactive Dyes	Reactive Black 5	10 000 – 100 000

Fourteen substances, including four of the seven substances in Table 4-1, were identified as being used in Canada in 2010 (see Table 4-2), based on information submitted by the Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers (ETAD) (personal communication, email from ETAD to Environment Canada, dated August 2010; unreferenced).

Table 4-2. List of Azo Direct Dyes and Reactive Dyes with recent commercial activity in Canada according to the Ecological and Toxicological Association of Dyes and Organics Pigments Manufacturers (personal communication, email from ETAD to Environment Canada, dated August 2010; unreferenced)

Subgroup	Name
Azo Direct Dyes	Direct Yellow 11
Azo Direct Dyes	Direct Green 28
Azo Direct Dyes	Direct Yellow 28
Azo Direct Dyes	Direct Yellow 12

Subgroup	Name
Azo Direct Dyes	Direct Yellow 50
Azo Direct Dyes	Direct Orange 26
Azo Direct Dyes	Direct Red 31
Azo Direct Dyes	Direct Violet 51
Azo Direct Dyes	Direct Red 81 triethanolamine salt
Azo Direct Dyes	Direct Orange 26
Azo Direct Dyes	Direct Orange 39
Azo Direct Dyes	Direct Blue 71
Azo Direct Dyes	Direct Black 56
Azo Reactive Dyes	Reactive Black 158

4.2 Uses

Azo Direct Dyes are generally used for colouring of paper, cotton, rayon, linen, jute, hemp, silk and polyamide textile materials and in batch dyeing of leather (HSDB 1983– ; Ishikawa et al. 2008; Kirk-Othmer 2010; CII 2013.

Azo Reactive Dyes are used primarily in the textiles industry for dyeing cellulosic fibres such as cotton and rayon, but can also be applied to wool, silk, polyamide and leather (Ishikawa et al. 2008; Kirk-Othmer 2010).

Table 4-3 presents a summary of major uses in Canada of certain substances in this Screening Assessment based on Consumer and Commercial Codes submitted in response to a recent survey issued pursuant to section 71 of CEPA 1999 (Canada 2011; Environment Canada 2012).

Table 4-3. Summary of the major uses of Reactive Black 5 and certain Azo Direct Dyes in Canada based on Consumer and Commercial Codes (indicated in parentheses) submitted in response to a section 71 survey (Canada 2011; Environment Canada 2012)

Substance	Ink, Toner and Colourants (C306)	Fabric, Textile and Leather Articles (C104)	Paper Products, Mixtures or Manufactured Items (C302)	Apparel and Footwear Care (C110)
Direct Blue 71	Yes	No	No	No
Direct Orange 26	Yes	No	No	No
Direct Orange 39	No	Yes	No	No
Direct Yellow 11	No	Yes	No	No
Direct Yellow 11 lithium salt	No	No	Yes	No
Direct Yellow 34	No	No	Yes	No
Reactive Black 5	Yes	Yes	No	Yes

In Canada, food colouring agents are regulated as food additives under the *Food and Drug Regulations* (Canada [1978]). Colours that are permitted for use in food are included in the *List of Permitted Colouring Agents*, incorporated by reference in the *Marketing Authorization for Food Additives that May be Used As Colouring Agents*, issued under the authority of the *Food and Drugs Act* (Canada 1985). None of the 69 substances identified in this Screening Assessment are included on the *List of Permitted Colouring Agents* as a permitted food colourant.

Two Azo Direct Dyes were identified for use in food packaging applications in Canada. Direct Blue 71 was identified as a component of inks and dyes for food packaging materials. Direct Yellow 11 lithium salt was identified as a component of a colourant that may be used in paperboard material to package food (personal communications, emails from the Food Directorate [Health Canada] to the Risk Management Bureau [Health Canada], dated 2011; unreferenced).

Colouring agents permitted to be used in drugs in Canada are regulated under Part C, Division 1, of the *Food and Drug Regulations* (Canada [1978]). None of the substances in this Screening Assessment are listed as a permitted colouring agent in drugs, nor have any been identified to be present in pharmaceuticals, veterinary drugs or biologics in Canada (personal communication, email from the Therapeutic Products Directorate [Health Canada] to the Risk Management Bureau [Health Canada], dated 2011; unreferenced; personal communication, email from the Veterinary Drugs Directorate [Health Canada] to the Risk Management Bureau [Health Canada], dated 2011; unreferenced; personal communication, email from the Biologics and Genetic Therapies Directorate [Health Canada] to the Risk Management Bureau [Health Canada], dated 2011; unreferenced).

None of the Azo Direct Dyes or Azo Reactive Dyes are listed in the Natural Health Products Ingredients Database (NHPID) as ingredients for use in natural health products and are not listed in the Licensed Natural Health Products Database to be present in currently licensed natural health products (NHPID, 2011; LNHPD, 2011).

Based on notifications submitted under the *Cosmetic Regulations* to Health Canada, none of the substances in this Screening Assessment are used in certain cosmetic products in Canada (personal communication, email from the Consumer Product Safety Directorate [Health Canada] to the Existing Substances Risk Assessment Bureau [Health Canada], dated 2011; unreferenced). These substances are not included on the List of Prohibited and Restricted Cosmetic Ingredients (more commonly referred to as the Cosmetic Ingredient Hotlist or simply the Hotlist), 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 2011b).

None of the Azo Direct Dyes or Azo Reactive Dyes was identified for use as a formulant in pest control products registered in Canada (personal communication, email from the Pest Management Regulatory Agency [Health Canada] to the Risk Management Bureau [Health Canada], dated 2011; unreferenced).

In addition, no uses of substances in this Screening Assessment for military applications in Canada were identified (personal communication, email from the Department of National Defence to the Risk Management Bureau [Health Canada], dated 2011; unreferenced).

5. Environmental Fate and Behaviour

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

As explained in Environment Canada and Health Canada (2013), mass balance fate models, such as the Equilibrium Criterion model (New EQC 2011), are not applicable for strongly ionic azo (e.g., direct and reactive) dyes, and their use with these substances would not conform to good modelling practice for multimedia models (Buser et al. 2012). Therefore, the environmental fate and compartmentalization of these substances are discussed qualitatively using information on their physical and chemical properties.

5.1 Releases to Water and Sediment

Azo Direct Dyes and Azo Reactive Dyes are highly water soluble and have an inherently high affinity for cellulosic substrates, which results in high fixation rates (ETAD 1995; Environment Canada and Health Canada 2013). Given a continuous steady-state emission to water, a large proportion of ionic dyes will reside in the water column due to their high water solubility. Over time, Azo Direct and Azo Reactive Dyes could bind to suspended organic matter via electrostatic interactions and eventually settle out to bed sediments or wastewater sludge (ETAD 1995).

Other factors, such as increasing molecular size, water hardness and salinity as well as decreasing pH, are thought to support 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.

5.2 Releases to Soil

Azo Direct and Azo Reactive Dyes may be released indirectly to soil via the application of biosolids to agricultural land. Azo Direct and Azo Reactive Dyes have an inherently high affinity for positively charged substrates, with relatively high polarity and high recalcitrance. Recalcitrance is difficult to evaluate because of the dependence of degradation on highly variable boundary conditions (e.g., pH) (Zaharia and Suteu 2012).

5.3 Releases to Air

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

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

5.4 Environmental Persistence

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

5.4.1 Empirical Data for Persistence

Empirical biodegradation data related to the persistence of Azo Direct and Azo Reactive Dyes are limited.

5.4.1.1 Aerobic Biodegradation

Limited experimental data for degradation under aerobic conditions in the absence of microorganisms were found for substances in the Azo Direct and Azo Reactive Dye grouping (Tables 5-1 and 5-2). The majority of the experimental data shown in these tables were obtained either in the presence of bacteria or under a modified OECD protocol. The modified protocol makes it difficult to compare the results with those of standard OECD Test Guideline 301C tests, since sludge was acclimated to the dyes, and longer test periods were used. Also, it is unclear whether commercial formulations were used in some of these tests instead of high-purity dyes.

Senan and Abraham (2004) observed that an aerobic bacterial consortium consisting of two isolated strains (BF1, BF2) and a strain of *Pseudomonas putida* (MTCC1194) could effectively break down a mixture of azo dyes in very limited quantities (under laboratory conditions).

Table 5-1. Selected experimental data for biodegradation of Azo Direct Dyes under aerobic conditions with bacteria and controlled environmental settings

Substance (CAS RN)	Method	Degradation value (%, except where noted)[a]	Degradation endpoint	Test duration (d)	Reference
Direct Orange 39 (1325-54-8)	Neelambari et al. 2013	< 75	Half-life	92	Neelambari et al. 2013
Direct Yellow 12 (2870-32-8)	Modified OECD 301E biodegradation test	< 25	DOC degradation	28	Brown and Hamburger 1987
Direct Blue 71 (4399-55-7)	Modified OECD 301E biodegradation test	25–75	DOC degradation	42	Pagga and Brown 1986

Abbreviations:; DOC, dissolved organic carbon; OECD, Organisation for Economic Co-operation and Development

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

Table 5-2. Selected experimental data for biodegradation of Azo Reactive Dyes under aerobic conditions with bacteria and controlled environmental settings

Substance (CAS RN)	Method	Degradation value (%, except where noted)[a]	Degradation endpoint	Test duration (d)	Reference
Reactive Black 5 (17095-24-8)	Modified OECD 301E biodegradation test	100	DOC degradation	28	Pagga and Brown 1986
Reactive Black 5 (17095-24-8)	Modified OECD 301E biodegradation test	75–100	DOC degradation	28	Øllgaard et al. 1998

Abbreviations: DOC, dissolved organic carbon; OECD, Organisation for Economic Co-operation and Development

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

5.4.1.2 Anaerobic Biodegradation

Under anaerobic or reducing conditions, biotic degradation of dyes may take place relatively rapidly (Yen et al. 1991; Baughman and Weber 1994; ETAD 1995; 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). Select experimental data (Tables 5-3 and 5-4) under anaerobic conditions showed significant degradation. Differences observed in degradation value percentages are due to the different bacterial strains and laboratory parameters (i.e., pH, salinity and temperature).

Table 5-3. Selected experimental data for biodegradation of Azo Direct Dyes under anaerobic conditions

Substance (CAS RN)	Method	Degradation value (%)[a]	Degradation endpoint	Test duration (d)	Reference
Direct Orange 39 (1325-54-8)	Brown and Laboureur 1983	90–95	Colour removal	3	ETAD 2008
Direct Orange 39 (1325-54-8)	Brown and Laboureur 1983	95	Colour removal	7	ETAD 2008
Direct Yellow 12 (2870-32-8)	Brown and Laboureur 1983	100	Colour removal	7	Brown and Laboureur 1983

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

Table 5-4. Selected experimental data for biodegradation of Azo Reactive Dyes under anaerobic conditions

Substance (CAS RN)	Method	Degradation value (%)[a]	Degradation endpoint	Test duration (d)	Reference
Reactive Black 5 (17095-24-8)	Carliell et al. 1994	80–85	Colour removal	4	Carliell et al. 1994
Reactive Black 5 (17095-24-8)	Brown and Laboureur 1983	60	Colour removal	28	Pagga and Brown 1986
Reactive Black 5 (17095-24-8)	NA	72	Colour removal	28	ETAD 2008

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

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 (2010) models used, thereby increasing the reliability of the predictions. Given the ecological relevance of the water compartment, the fact that most of the available models apply to water and the fact that Azo Direct and Azo Reactive Dyes are expected to be released to this compartment, aerobic biodegradation in water was primarily examined.

Tables A7–A10 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 Sub-models 3–6 (BIOWIN 2010), DS TOPKAT (©2005–2009) and CATALOGIC (2012).

All of the model outputs for Azo Direct and Azo Reactive Dyes (other than a few substances using BIOWIN Sub-models 3 and 4) consistently predicted that these substances would biodegrade very slowly in water under aerobic conditions (Appendix A, Tables A7–A10). 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.

5.4.3 Hydrolysis

The majority of the Azo Direct Dyes do not contain functional groups expected to undergo hydrolysis. The substances in the assessment have non-benzidine presence; some substances have one or two naphthalene rings, with the majority containing highly soluble terminal salt groups. This is consistent with published studies that note hydrolysis as being an insignificant factor in the cleavage of azo compounds (Baughman and Perenich 1988).

Only one of the eight Azo Reactive Dyes appears to have hydrolysis potential (Reactive Black 5). Reactive dyes form covalent bonds with the textile; therefore, the fixation competes with the reaction of the leaving group of the reactive dye with water (hydrolysis).

5.4.4 Summary of Persistence

On the basis of the slow degradation and lack of hydrolysis of Azo Direct and Azo Reactive Dyes in aerobic environments in combination with their 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 be dispersed in the aquatic environment. In sediment and soil, biodegradation is also expected to be slow under aerobic conditions and relatively fast under anaerobic conditions. Air is not considered to be a medium of concern due to a lack of releases and partitioning to air; furthermore, these substances are not expected to undergo long-range atmospheric transportation.

5.5 Potential for Bioaccumulation

In this assessment, a variety of lines of evidence have been used to determine the bioaccumulation potential of Azo Direct and Azo Reactive Dyes. 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 Azo Direct and Azo Reactive Dyes, since these substances are outside the model domains of applicability.

5.5.1 Octanol–Water Partition Coefficient

As indicated in Tables 3-1 to 3-4, Azo Direct and Azo Reactive Dyes as subgroups have relatively high water solubility (0.02–190 g/L Azo Direct; 40–200 g/L Azo Reactive). No experimental log K_{ow} was found for either subgroup. Available literature for these substances suggests that they would have very low log K_{ow} values (ETAD 1995), which would also suggest a very low bioaccumulation potential, according to equilibrium partitioning theory.

5.5.2 Aquatic Bioconcentration Factors

Empirical BCFs were determined to be < 10 for Reactive Black 5 (Anliker et al. 1981), indicating that Azo Reactive Dyes are not likely to bioconcentrate in aquatic organisms. No experimental data were found for the Azo Direct Dyes subgroup.

5.5.3 Other Factors for Assessing Bioaccumulation Potential

As outlined in the “Potential for Bioaccumulation” section of Environment Canada and Health Canada (2013), due to the lack of empirical bioaccumulation data available for Azo Direct Dyes and the very limited data available for Azo Reactive Dyes, 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 from exposures of organisms to these substances in soil and sediment are limited, mostly due to their high water solubility (Environment Canada and Health Canada 2013).

In general, Azo Direct Dyes are relatively hydrophilic, large molecules with high molecular weights (most above 500 g/mol). The minimum and maximum cross-sectional diameters for Azo Direct Dyes range from 0.89 nm (D_{min}) to 1.85 nm (D_{max}) (Tables 3-1 to 3-3). The small cross-sectional diameters of these substances would likely not be a factor in restricting their rate of uptake when crossing cell membranes; however, their high molecular weights may slow their uptake into biological organisms. The minimum and maximum cross-sectional diameters for Azo Reactive Dyes range from 1.00 nm (D_{min}) to 1.56 nm (D_{max}) (Table 3-4). The small to moderate cross-sectional diameters of these substances would likely not be a factor in restricting their rate of uptake when crossing cell membranes.

Results of mechanistic profiling for binding using version 3.1 of the OECD QSAR Toolbox (2012) suggest that some of these structures (e.g., those with non-hindered nitro or amine groups or benzyl halides) can potentially undergo covalent interactions with biological substrates (nucleophilic substitution reactions, Michael-type addition reactions, acylation, etc.) and bind with deoxyribonucleic acid (DNA) and proteins. In fact, some ionic dyes have been shown to have moderate to high levels of bioaccumulation and toxicity potential in soil organisms from protein binding to dermal surfaces of earthworms causing respiratory failure due to similar mechanistic reactions identified above (Princz et al. 2013). Biota–soil accumulation factors have been found to be > 10 in some cases, with little or no depuration of the bound chemical from the organism (Princz et al. 2013). Since no empirical bioaccumulation data for sediment or soil organisms are available for the dyes assessed here, the potential bioaccumulation from binding mechanisms in soil or sediment biota cannot be addressed in this assessment. Potential for adverse effects on organisms is discussed further in Section 6.

5.5.4 Summary of Bioaccumulation Potential

Azo Direct and Azo Reactive Dyes are expected to have a low bioaccumulation potential in aquatic organisms due primarily to lack of partitioning from water into aquatic organisms. This is supported by and consistent with their physical and chemical properties (i.e., low log K_{ow}, ionized at relevant environmental pH and high water solubility).

6. Potential to Cause Ecological Harm

6.1 Ecological Effects Assessment

Only empirical data on specific substances within the subgroups have been considered for the assessment of the ecological effects potential of Azo Direct and Azo Reactive Dyes given the high level of uncertainty associated with modelling the ecotoxicity of these substances.

6.1.1 Empirical Studies for the Aquatic Compartment

Available experimental data from aquatic toxicity studies for certain Azo Direct and Azo Reactive Dyes are presented in Tables 6-1 and 6-2, respectively. Most of the studies were acute toxicity studies addressing lethality. For most substances, there were few empirical data, whereas for other substances, such as Reactive Black 5, there were multiple aquatic toxicity studies. Regardless, sufficient toxicity data were available to consider three subsets (Monoazo, Disazo and Polyazo) for the Azo Direct Dyes subgroup and one subgroup representing the eight Azo Reactive Dyes.

Several key data points originated from an empirical toxicity study on the acute toxicity of 46 dyes to fathead minnow (*Pimephales promelas*), which is described in two scientific articles: first in Little and Lamb (1973), and then summarized in Little et al. (1974). While this publication is not recent, the study was carried out according to published standard methods, 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. This was originally reported in the two studies as a median tolerance limit (TL_{50}), but may also be interpreted as a median lethal concentration (LC_{50}).

Two experimental toxicity studies were found in the literature representing the Monoazo Direct Dye subset (Table 6-1). One study was an acute (48-hour) exposure where an LC_{50} of 180 mg/L was calculated for fathead minnow (*Pimephales promelas*) exposed to Direct Yellow 11 (Little et al. 1974). The other study involved 48-h exposure of Daphnia magna to Direct Yellow lithium salt (LC_{50} > 100). For the Disazo Direct Dyes, more toxicity data were available. Data were found for multiple fish species. Acute toxicity studies reported LC_{50} and LC_{100} values ranging from 125 to 1700 mg/L. Only one study was available for the Polyazo Direct Dye subset, in the form of a 48-hour LC_{50} of 75 mg/L using rainbow trout (*Onchorynchus mykiss*) exposed to the analogue, Direct Black 30 (Study Submission 2007).

Five fish toxicity studies were found for the Azo Reactive Dye subgrouping. Acute values (48- and 96-hour LC_{50}s) varied from > 100 to 1000 mg/L when fish species were exposed to Reactive Black 5. One chronic study (14 days) exposing *Oryzias latipes* to Reactive Black 5 was found (Table 6-2).

While the sensitivity of daphnids to Azo Direct Dyes was similar to that of various fish species, daphnids exposed to Azo Reactive Dyes were found to be more sensitive than fish. Daphnid reproduction was found to be the most sensitive endpoint, with a 21-day no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC) of 1.25 and 2.5 mg/L, respectively, when exposed to Reactive Black 5 (ECHA 2013).

Table 6-1. Selected empirical data for aquatic toxicity from representative substances for the Azo Direct Dyes subgroup.

Subgroup (subset)	Test organism	Type of test (duration)	Endpoint	Value (mg/L) (substance)	Reference
Azo Direct Dyes (Monoazo)	Fish *Pimephales promelas*	Acute (48 h)	LC_{50}	180[a] (Direct Yellow 11)	Little et al. 1974
Azo Direct Dyes (Monoazo)	Daphnid *Daphnia magna*	Acute (48 h)	LC_{50}	> 100 (Direct Yellow 11 lithium salt)	BASF 2004
Azo Direct Dyes (Disazo)	Fish *Oncorhynchus mykiss*	Acute (48 h)	LC_{50}	550 (Direct Yellow 34)	Personal communication email from Clariant, 2006 (unreferenced)
Azo Direct Dyes (Disazo)	Fish *Pimephales promelas*	Acute (24 h)	LC_{50}	180 (Direct Yellow 12)	Little et al. 1974
Azo Direct Dyes (Disazo)	Fish *Pimephales promelas*	Acute (48 h)	LC_{50}	130 (Direct Yellow 12)	Little et al. 1974
Azo Direct Dyes (Disazo)	Fish *Pimephales promelas*	Acute (96 h)	LC_{50}	125[a] (Direct Yellow 12)	Little and Lamb 1973
Azo Direct Dyes (Disazo)	Fish *Oryzias latipes*	Acute (96 h)	LC_{50}	600 (Direct Yellow 50)	CHRIP ©2008
Azo Direct Dyes (Disazo)	Fish *Leuciscus idus*	Acute (48 h)	LC_{100}	1400 (Direct Yellow 50)	Hamburger et al. 1977
Azo Direct Dyes (Disazo)	Fish *Oncorhynchus mykiss*	Acute (48 h)	LC_{100}	> 500 (Direct Yellow 50)	Hamburger et al. 1977
Azo Direct Dyes (Disazo)	Fish *Oncorhynchus mykiss*	Acute (48 h)	LC_{50}	> 1000 (Direct Yellow 50)	Hamburger et al. 1977

Subgroup (subset)	**Test organism**	**Type of test (duration)**	**Endpoint**	**Value (mg/L) (substance)**	**Reference**
Azo Direct Dyes (Disazo)	Fish *Oncorhynchus mykiss*	Acute (48 h)	LC_{50}	> 1400 (Direct Yellow 50)	Hamburger et al. 1977
Azo Direct Dyes (Disazo)	Fish *Leuciscus idus*	Acute (48 h)	LC_{50}	125 (Direct Yellow 50)	Little and Lamb 1972
Azo Direct Dyes (Disazo)	Fish *Phoxinus phoxinus*	Acute (48 h)	LC_{50}	> 1000 (Direct Yellow 50)	CHRIP ©2008
Azo Direct Dyes (Disazo)	Fish *Phoxinus phoxinus*	Acute (48 h)	LC_{50}	1700 (Direct Yellow 50)	CHRIP ©2008
Azo Direct Dyes (Disazo)	Fish *Pimephales promelas*	Acute (48 h)	LC_{50}	> 180 (Direct Yellow 50)	Little et al. 1974
Azo Direct Dyes (Disazo)	Fish *Pimephales promelas*	Acute (24 h)	LC_{50}	> 180 (Direct Yellow 50)	Little et al. 1974
Azo Direct Dyes (Disazo)	Fish *Pimephales promelas*	Acute (96 h)	LC_{50}	> 180 (Direct Yellow 50)	Little and Lamb 1973
Azo Direct Dyes (Disazo)	Fish *Oncorhynchus mykiss*	Acute (48 h)	LC_{50}	180 (Direct Yellow 50)	Personal communication email from Clariant, 2006 (unreferenced)
Azo Direct Dyes (Polyazo)	Fish *Oncorhynchus mykiss*	Acute (48 h)	LC_{50}	75[a] (Direct Black 30)	Study Submission 2007

Abbreviations: LC_{50}, the concentration of a substance that is estimated to be lethal to 50% of the test organisms; LC_{100}, the concentration of a substance that is estimated to be lethal to 100% of the test organisms

[a] Selected critical toxicity value.

Table 6-2. Selected empirical data for aquatic toxicity from representative substances for the Azo Reactive Dyes subgroup.

Subgroup	**Test organism**	**Type of test (duration)**	**Endpoint**	**Value (mg/L) (substance)**	**Reference**
Azo Reactive Dyes	Waterflea *Moina macrocopa*	Acute	EC_{50}	18.2 (Reactive Red 141)	Vinitnantharat et al. 2008
Azo	Algae	Acute	IC_{50}	95.5	Vinitnantharat

Subgroup	**Test organism**	**Type of test (duration)**	**Endpoint**	**Value (mg/L) (substance)**	**Reference**
Reactive Dyes	*Chlorella* sp.			(Reactive Red 141)	et al. 2008
Azo Reactive Dyes	Fish *Oryzias latipes*	Acute (48 h)	LC_{50}	> 100 (Reactive Black 5)	ECHA 2013
Azo Reactive Dyes	Fish *Leuciscus idus melanotus*	Acute (96 h)	LC_{50}	> 100 (Reactive Black 5)	ECHA 2013
Azo Reactive Dyes	Fish *Leuciscus idus melanotus*	Acute (48 h)	LC_{50}	> 500 (Reactive Black 5)	ECHA 2013
Azo Reactive Dyes	Fish *Brachydanio rerio*	Acute (96 h)	LC_{50}	> 500 (Reactive Black 5)	ECHA 2013
Azo Reactive Dyes	Fish *Brachydanio rerio*	Chronic (28 d)	LC_{10}	100 (Reactive Black 5)	ECHA 2013
Azo Reactive Dyes	Daphnia *Daphnia magna*	Acute (48 h)	EC_{50} mobility	748 (Reactive Black 5)	ECHA 2013
Azo Reactive Dyes	Daphnia *Daphnia magna*	Acute (48 h)	EC_{50} mobility	72.9 (Reactive Black 5)	ECHA 2013
Azo Reactive Dyes	Daphnia *Daphnia magna*	Acute (48 h)	EC_{50} mobility	> 800 (Reactive Black 5)	ECHA 2013
Azo Reactive Dyes	Daphnia *Daphnia magna*	Acute (48 h)	EC_{50} mobility	750 (Reactive Black 5)	ECHA 2013
Azo Reactive Dyes	Daphnia *Daphnia magna*	Acute (48 h)	EC_{50} mobility	> 128 (Reactive Black 5)	ECHA 2013
Azo Reactive Dyes	Daphnia *Daphnia magna*	Acute (48 h)	EC_{100} mobility	> 128 (Reactive Black 5)	ECHA 2013
Azo Reactive Dyes	Daphnia *Daphnia magna*	Acute (48 h)	EC_{50} reproduction	> 20 (Reactive Black 5)	ECHA 2013
Azo Reactive Dyes	Daphnia *Daphnia magna*	Chronic (21 d)	LOEC reproduction	2.5 (Reactive Black 5)	ECHA 2013

Subgroup	Test organism	Type of test (duration)	Endpoint	Value (mg/L) (substance)	Reference
Azo Reactive Dyes	Fish *Oryzias latipes*	Acute (48 h)	LC_{50}	1000 (Reactive Black 5)	MITI 1992
Azo Reactive Dyes	Fish *Danio rerio*	Acute (96 h)	LC_{50}	500 (Reactive Black 5)	ETAD 2008
Azo Reactive Dyes	Fish *Oryzias latipes*	Acute (96 h)	LC_{50}	> 100 (Reactive Black 5)	CHRIP ©2008
Azo Reactive Dyes	Fish *Oryzias latipes*	Chronic (14 d)	LC_{50}	> 100 (Reactive Black 5)	CHRIP ©2008
Azo Reactive Dyes	Fish *Oryzias latipes*	Acute (48 h)	LC_{50}	> 100 (Reactive Black 5)	CHRIP ©2008
Azo Reactive Dyes	Fish *Oryzias latipes*	Acute (48 h)	LC_{50}	> 100 (Reactive Black 5)	CHRIP ©2008
Azo Reactive Dyes	Algae *Pseudokirchneriella subcapitata*	Acute	IC_{50}	20.5 (Reactive Black 5)	Visani de Luna 2012
Azo Reactive Dyes	Daphnid *Daphnia similis*	Acute	EC_{50}	37.5 (Reactive Black 5)	Visani de Luna 2012
Azo Reactive Dyes	Daphnid *Ceriodaphnia dubia*	Chronic reproduction	IC_{50}	24.6 (Reactive Black 5)	Visani de Luna 2012
Azo Reactive Dyes	Daphnid *Ceriodaphnia dubia*	Chronic reproduction	NOEC	10 (Reactive Black 5)	Visani de Luna 2012

Abbreviations: EC_{50}, effective concentration for 50% of test organisms; EC_{100}, effective concentration for 100% of test organisms; IC_{50}, median inhibitory concentration; LC_{10}, lethal concentration for 10% of test organisms; LC_{50}, lethal concentration for 50% of test organisms; LOEC, lowest-observed-effect concentration; NOEC, no-observed-effect concentration

6.1.2 Empirical Studies for Other Environmental Compartments

No ecotoxicological data for sediment- or soil-dwelling organisms were found for the Azo Direct or Azo Reactive Dye subgroups.

6.2 Derivation of the Predicted No-Effect Concentration

6.2.1 Derivation of the Aquatic Predicted No-Effect Concentration

A grouping (read-across) approach was used in the development of an aquatic predicted no-effect concentration (PNEC) for each of the Azo Direct Dye and Azo Reactive Dye subgroups. No distinction was made for each of the Azo Direct Dye subsets (Monoazo, Disazo and Polyazo), given the similar ranges in aquatic toxicity among the three subsets.

The lowest endpoint value available from the most sensitive organism identified for each subgroup was selected as the critical toxicity value (CTV) representing the subgroup. From this the lowest of the three CTVs was chosen to represent the subgroup. For the Azo Direct Dyes, the aquatic CTV selected was a 48-hour LC_{50} of 75 mg/L obtained for rainbow trout (*Oncorhynchus mykiss*) exposed to the Polyazo analogue, Direct Black 30 (Study Submission 2007). 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-effect concentration from a short-term LC_{50}). Therefore, a PNEC of 0.75 mg/L was calculated for the Azo Direct Dyes.

For the Azo Reactive Dyes, the aquatic CTV selected was a chronic 21-day LOEC for reproduction of 2.5 mg/L for *Daphnia magna* (ECHA 2013). This was the most sensitive valid experimental value. The aquatic PNEC was then derived by dividing this value by an assessment factor of 10 (to account for differences in interspecies and intraspecies variability). Therefore, a PNEC of 0.25 mg/L was calculated for the Azo Reactive Dyes.

6.2.2 Ecological Effects Summary

Based on lines of evidence involving empirical and read-across aquatic ecotoxicity data, it may be concluded that some Monoazo, Disazo and Polyazo Direct Dyes may cause harm to aquatic organisms at moderate concentrations (i.e., LC_{50}s are < 100 mg/L). In addition, it is expected that Azo Reactive Dyes may cause harm to aquatic organisms at low concentrations (i.e., 21-day NOEC and LOEC of 1.25 and 2.5 mg/L, respectively).

6.3 Ecological Exposure Assessment

6.3.1 Releases to the Environment

No measured environmental concentrations (in air, water, soil or sediment) of Azo Direct or Azo Reactive Dyes in Canada have been identified. Environmental concentrations have therefore been estimated from available information.

Anthropogenic releases of a substance to the environment depend upon various losses that occur during the manufacture, industrial, consumer or commercial[3] use and disposal of the substance. In order to estimate releases to the environment occurring at different stages of the life cycle of the Azo Direct and Azo Reactive Dyes, Environment Canada compiled information on the relevant sectors and product lines as well as emission factors[4] to wastewater, land and air at different life cycle stages in order to identify the life cycle stages that are the largest contributors to environmental concentrations. Recycling activities and transfer to waste disposal sites (landfill, incineration) were also considered. However, releases to the environment from disposal were not quantitatively accounted for unless reliable specific information on the rate of (or potential for) release from landfills and incinerators was available.

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

6.3.2 Identification of Important Ecological Exposure Scenarios

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

Azo Direct and Azo Reactive Dyes are not manufactured in Canada, according to the data collected from a 2011 regulatory survey for aromatic azo and benzidine-based substances (Canada 2011).

For reporting year 2010, six Azo Direct Dyes were imported in a combined total quantity between 10 000 kg and 100 000 kg, and one Azo Reactive Dye was imported within the same quantity range, according to the survey data. Some of these dyes were

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[3] Commercial use is the use of a chemical substance, or the use of a mixture, product or manufactured item containing a chemical substance, in a commercial enterprise providing saleable goods or services.

[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 a life cycle stage, such as manufacture, processing, industrial application or commercial/consumer use. Sources of emission factors include emission scenario documents developed under the auspices of the OECD, data reported to Environment Canada's National Pollutant Release Inventory, industry-generated data and monitoring data.

formulated before being distributed to their end users. Azo Direct Dyes were used primarily for paper dyeing (98.7%). The use of the Azo Direct Dyes for textile dyeing and cleaning products was very limited (1.1% and 0.3%, respectively). The in-commerce Azo Reactive Dye was used exclusively for textile dyeing.

Based on the above use patterns, the following three scenarios are identified as the major potential sources of environmental releases:

1. Chemical formulation (involving both Azo Direct and Azo Reactive Dyes)
2. Paper dyeing (involving Azo Direct Dyes only)
3. Textile dyeing (involving Azo Reactive Dyes only)

Deinking is not considered to be a major potential source of environmental releases. Although paper products dyed with the Azo Direct Dyes can be recycled at deinking mills, the dyes are destroyed during bleaching operations (Hannuksela and Rosencrance 2008; Huber and Carré 2012). Thus, the environmental releases of dyes from deinking are expected to be negligible.

The consumer and/or commercial uses of textile products are a potential source of environmental releases. Since the uses of these products are more dispersive than textile dyeing, their environmental concentrations are expected to be lower than those from industrial operations, and therefore no quantitative exposure assessment was conducted.

6.3.3 Estimates for Predicted Environmental Concentrations

The water column is considered to be an important environmental compartment for the presence of the direct and reactive dyes. These dyes enter the environment primarily via aqueous discharges from publically owned wastewater treatment systems to surface water. They have high water solubilities (in the range of 0.02–190 g/L) and are therefore expected to remain in significant quantities in the aqueous phase during wastewater treatment as well as in the water column upon release to surface water. Although these dyes are completely ionized in water and have the potential of being sorbed to wastewater sludge and sediment via electrostatic binding, the extent of this sorption cannot be quantified due to the lack of reliable estimation methods. As a result, the PEC calculations are limited to the aquatic compartment based on conservative estimates for wastewater treatment removal. PECs in soil resulting from land application of biosolids and those in sediment resulting from water to sediment partitioning were not calculated.

Each of the three scenarios is generic in nature and consists of multiple facilities located across various sites. These facilities are identified as the industrial users of dyes from various sources, including survey data. They include facilities involved with the direct and/or reactive dyes as well as facilities involved with other dyes. The inclusion of the latter in each scenario is intended to account for the variability in the overall sector (not only for the period for which survey data were obtained), the uncertainty associated with incomplete data and the likelihood of a switch-over from other dyes to the direct and/or reactive dyes.

The aquatic PECs for the three scenarios or sectors are estimated for receiving water near discharge points. The PECs are calculated using a probabilistic estimation method that relies on information compiled for each sector as a whole and integrates different possible values for the selected parameters (e.g., those that are known to vary or fall within a certain range). As a result of this method, the aquatic PECs are derived as a probabilistic distribution for a sector/scenario.

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

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

where:

PEC: aquatic predicted environmental concentration (μg/L)

Q: daily use quantity of direct and/or reactive dyes at a facility (kg/d)

E: emission factor of direct or reactive dyes to process wastewater prior to any wastewater treatment (%)

R: removal of direct or reactive dyes by industrial and/or publically-owned wastewater treatment systems (%)

V: daily dilution water volume near the discharge point of an industrial or publicaly-owned wastewater treatment system (L/d)

10^9: conversion factor from kg to μg

For paper dyeing, the following equation is used:

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

where:

PEC: aquatic predicted environmental concentration (μg/L)

q: use rate of direct dyes (kg of dyes per tonne of paper produced, or kg/t)

E: emission factor of direct dyes to process wastewater prior to any wastewater treatment (%)

R: removal of direct dyes by industrial and/or publicaly-owned wastewater treatment systems (%)

v: per tonne dilution water volume near the discharge point of an industrial or publically-owned wastewater treatment system (L per tonne of paper produced, or L/t)

10^9: conversion factor from kg to µg

For a given sector/scenario, the daily use quantity (Q) or use rate (q) and emission factor (E) are each determined to be in a certain range that is intended to be applicable to all facilities within the sector as well as to all possible operating conditions at a single facility. The removal by wastewater treatment systems (R) is a result of pollution mitigation on-site, off-site or both before the treated wastewater is released to the aquatic environment. This removal is determined as a range for the direct dyes under secondary or lagoon treatment due to a wide range of biodegradation data found in the literature. In contrast, the removal is determined as a single value for the direct dyes under primary treatment as well as for the reactive dyes under primary, secondary or lagoon treatment. The daily dilution water volume (V) or the per tonne dilution water volume (v) is derived based on several parameters, including daily or per tonne wastewater volume from an industrial facility, wastewater flow from a publically-owned wastewater treatment system and the dilution factor of the receiving water near the discharge point of an industrial or publically-owned wastewater treatment system.

The removal by wastewater treatment (R) and the daily or per tonne dilution water volume (V or v) are characteristic of a site. In general, a sector/scenario consists of multiple sites, and the removal and the daily or per tonne dilution water volume are determined as discrete distributions (sets of data points). In some cases, only one site is identified for a sector/scenario, so a discrete distribution is reduced to a single data point.

Table 6-3 provides a summary of the ranges of parameter values determined for each of the three sectors/scenarios identified. Note that a proper range was not found for the use rate of direct dyes applicable to paper dyeing, and only a single value was used in the calculations.

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

Sector/scenario	Chemical formulation	Paper dyeing	Textile dyeing
Number of sites	3	15	24
Number of facilities	3	17	42
Dye daily use quantity (kg/d)	150–500	N/A	9.1–36.3
Dye use rate (kg/t)	N/A	0.1	N/A
Emission factor to wastewater (%)	0.5–1.4	2–21	3–40

Removal by wastewater treatment (%)	29.4–80.5	29.6–96.2	0.5–80.5
Daily dilution water volume (million L/d)	298–4970	N/A	7.4–3934
Per tonne dilution water volume (million L/t)	N/A	0.06–64	N/A

Crystal Ball, a commercial software program, was used to derive an aquatic PEC probabilistic distribution from the equation and the range of values determined for each parameter. A vast number of PECs were calculated by varying each parameter within the determined range (20 000 – 100 000 data points used for each range). These PECs represent different levels of exposure resulting from different conditions across all sites within a sector/scenario, and these different conditions are characterized by daily use quantity, emission factor, removal by wastewater treatment and daily or per tonne dilution water volume. The PECs derived were then sorted by their magnitudes and plotted as a function of the cumulative percentage or probability of occurrence. Such a plot is referred to as a cumulative probabilistic distribution. The three probabilistic distributions are presented in Table 6-4.

Table 6-4. Aquatic PECs for Azo Direct and Azo Reactive Dyes for three key industrial exposure scenarios

Percentile	Chemical formulation (µg/L)	Paper dyeing (µg/L)	Textile dyeing (µg/L)
0th	0.0	0.2	0.1
5th	0.1	1.2	0.4
10th	0.2	1.9	0.6
25th	0.4	3.8	1.2
50th	2.4	8.0	3.4
75th	4.4	15.8	8.3
90th	6.7	28.6	19.9
95th	7.9	54.1	40.3
100th	15.8	194.2	189.2

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

6.4 Characterization of Ecological Risk

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

6.4.1 Aquatic Probabilistic Risk Analysis

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

For the aquatic environment, a PNEC of 750 µg/L derived for Azo Direct Dyes (see Section 6.1) was compared with the PECs outlined in Section 6.3.3 (see Table 6-4) for sites using Azo Direct Dyes for paper dyeing. The probability of the PECs falling below the PNEC is 100% for the paper dyeing scenario. This high probability indicates that the chance of exceeding the PNEC is very low when the conditions of facilities and their related sites fall within the ranges given in Table 6-3.

The lower aquatic PNEC of 250 µg/L derived for Azo Reactive Dyes (see Section 6.1) was compared with the aquatic PECs outlined in Section 6.3.3 (see Table 6-4) for sites using Azo Reactive Dyes for textile dyeing. The probability of the PECs falling below the PNEC is 100% for this textile dyeing scenario. This high probability indicates that the chance of exceeding the PNEC is very low when the conditions of facilities and their related sites fall within the ranges given in Table 6-3.

Furthermore, the probability of the chemical formulation scenario PECs falling below the PNEC for Azo Direct or Azo Reactive Dyes is 100%.

6.4.2 Soil Risk Quotient Analysis

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

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

Azo Direct and Azo Reactive Dyes are not expected to occur naturally in the environment. No data concerning concentrations of these substances in the Canadian environment have been identified. Azo Direct and Azo Reactive Dyes are complex anionic molecules that generally have relatively high water solubilities (0.02 - 190 g/L) and are expected to dissociate at environmentally relevant pH levels. Since there is a relative paucity of data, Mono, Disazo and Polyazo Direct Dyes were examined as subsets with respect to their physical and chemical properties. The same grouping/read-across principles were applied for the eight substances in the Azo Reactive Dye subgroup. All Azo Direct and Azo Reactive Dyes were grouped according to their environmental fate based on their similar physical and chemical properties as well as relatively similar chemical structures (e.g., sharing common functional groups, but varying in number). Due to their high water solubility and affinity for oppositely charged organic particles, Azo Direct and Azo Reactive Dyes are expected to be found in water and may eventually settle out in sediment and soil. Given their 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.

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 1995). Azo Direct and Azo Reactive Dyes are not expected to bioconcentrate due to their very high molecular weights (> 500 g/mol) and relatively large minimum and maximum effective cross-sectional diameters, which suggest slow uptake potential. According to empirical and modelled data, Azo Direct and Azo Reactive Dyes are expected to biodegrade very slowly in aerobic environments and are therefore considered to be persistent in water, sediment and soil. However, Azo Direct and Azo Reactive Dyes may degrade and transform to certain aromatic amines if they reach anaerobic environments or via metabolic pathways if transferred from the diet into organisms.

Based on empirical aquatic ecotoxicity data, it is expected that Monoazo, Disazo and Polyazo Direct Dyes and Azo Reactive Dyes may cause harm to aquatic organisms at moderate to low concentrations (LC_{50}: 75 to > 1000 mg/L). Aquatic invertebrates (daphnids) were more sensitive than other organisms to Azo Reactive Dyes (NOEC for reproduction 1.25 mg/L). No toxicity data were available for terrestrial or sediment-dwelling organisms.

A conservative exposure analysis of the chemical formulation, paper dyeing and textile dyeing processes was done because those sectors were anticipated to present the highest potential ecological risk related to industrial releases to the environment for these substances. Using a probabilistic approach, the PECs were compared with the PNECs for water. The probability that the PECs of Azo Direct and Azo Reactive Dyes exceeded the PNECs was very low.

6.4.4 Uncertainties in Ecological Assessment

Many specific substances addressed in this report are data limited. This introduces some uncertainties, as there are still some degrees of structural variation between the substances assessed in the different 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, but available documentation is scarce. The use of assessment factors in determining a PNEC is intended to address this uncertainty. While water was found to be the key medium of interest, soil and sediment also hold some importance due to potential adsorption and electrostatic interactions. Therefore, the lack of available effects and bioaccumulation data for Azo Direct and Azo Reactive Dyes in soil and sediment is a source of uncertainty.

The lack of measured environmental concentrations of these substances (e.g., monitoring data) in Canada resulted in the need to evaluate risk based on predicted concentrations in water near industrial point sources. Conservative assumptions were made when using models to estimate concentrations in receiving water bodies.

Given the use of some of these substances in other countries, it is possible that they may enter the Canadian market as components of manufactured items and/or consumer products. However, it is anticipated that the proportions of these substances released to the various environmental media would not be significantly different from those estimated here, given the conservative assumptions used in the exposure analyses.

7. Potential to Cause Harm to Human Health

The human health assessment for Azo Direct Dyes and Azo Reactive Dyes focuses on 21 substances that are in commerce above the section 71 reporting threshold or for which available information indicates potential exposure to the general population of Canada. These substances are 18 of the 61 Azo Direct Dyes (Direct Black 56, Direct Blue 71, Direct Green 28, Direct Orange 26, Direct Orange 39, Direct Red 31, Direct Red 81 triethanolamine salt, Direct Violet 51, Direct Yellow 11, Direct Yellow 11 lithium salt, Direct Yellow 12, Direct Yellow 28, Direct Yellow 34, Direct Yellow 50, CAS RN 28706-21-0, CAS RN 71033-21-1, CAS RN 83221-56-1 and CAS RN 84878-17-1) and 3 of the 8 Azo Reactive Dyes (Reactive Black 5, Reactive Black 158 and Reactive Blue 225).

7.1 Exposure Assessment

7.1.1 Environmental Media and Food

Measured concentrations of Azo Direct Dyes and Azo Reactive Dyes in environmental media in Canada or elsewhere were not identified. Based on the uses and physical and chemical properties of the six Azo Direct Dyes and one Azo Reactive Dye that were imported into Canada, these substances are expected to partition into water rather than other environmental media. In one study, the presence of Reactive Black 5 was confirmed (not quantified) in raw textile wastewater from a textile dye house in Berlin by high-performance liquid chromatography coupled to tandem mass spectrometric detection (Storm et al. 2000). After anaerobic and aerobic treatment of the raw textile wastewater, analysis showed that Reactive Black 5 was no longer present. As discussed previously in Section 5.4.3, some portion of Reactive Black 5 is expected to be in the hydrolysed form if released to the environment. Overall, due to the very low volatility of these substances, their limited commercial quantities in Canada, their dispersive nature after release and expected removal by drinking water treatment, environmental media are not considered to be a significant source of exposure.

The Canadian Food Inspection Agency (CFIA) monitors the presence of certain colourants in foods, including five substances within these two subgroups: Direct Blue 71, Direct Violet 51, Direct Yellow 12, Direct Yellow 50 and Reactive Black 5. From 2009 to 2011, CFIA analyzed a total of 1646 samples of domestic and imported foods (including spices) in targeted food surveys. Samples were selected for their high likelihood of containing food colouring agents. None of the five substances listed above was detected in the two targeted food surveys at the level of detection of the monitoring system (CFIA 2010, 2011).

Although Direct Blue 71 and Direct Yellow 11 lithium salt are used in food packaging materials, the potential for these substances to migrate to food is anticipated to be minimal, and therefore exposure for the general population in Canada to these substances from food packaging materials is not expected to be significant (personal communications, emails from Food Directorate [Health Canada] to Risk Management Bureau [Health Canada], dated 2011; unreferenced).

7.1.2 Consumer Products

A variety of exposure scenarios are considered to be relevant to general population exposure in Canada, including use of textiles and leather products. Where substance-specific information was available, exposure estimates were derived for each substance. Otherwise, default parameters were applied (refer to Appendix B for details). Details of exposure estimates for all identified uses are provided in Appendix B.

Direct dyes may migrate from textile materials (BfR 2007). As such, there is potential exposure to 14 Azo Direct Dyes— Direct Black 56, Direct Green 28, Direct Orange 26, Direct Orange 39, Direct Red 31, Direct Red 81 triethanolamine salt, Direct Violet 51, Direct Yellow 12, Direct Yellow 28, Direct Yellow 50, CAS RN 28706-21-0, CAS RN 71033-21-1, CAS RN 83221-56-1 and CAS RN 84878-17-1—that are used as dyes in textiles. Estimated daily exposures of the general population to these substances via the dermal route from direct contact with textiles are presented in Table 7-1. Assumptions and default parameters for these scenarios are outlined in Appendix B1. Estimates of oral exposure as a result of mouthing of textiles by infants are included in Table 7-1.

In addition to being used as textile dyes, nine Azo Direct Dyes—Direct Black 56, Direct Red 31, Direct Red 81 triethanolamine salt, Direct Yellow 12, Direct Yellow 50, CAS RN 28706-21-0, CAS RN 71033-21-1, CAS RN 83221-56-1 and CAS RN 84878-17-1—are also commonly used as dyes for leather, according to the Colour Index International database that is published jointly by the Society of Dyers and Colourists and the American Association of Textile Chemists and Colorists (CII 2013). Estimated exposures from direct skin contact with leather products are included in Table 7-1 (refer to Appendix B2 for more details).

Direct Blue 71, Direct Red 31, Direct Yellow 11, Direct Yellow 11 lithium salt, Direct Yellow 12, Direct Yellow 34 and Direct Yellow 50 are used to dye paper (CII 2013; Environment Canada 2012). Although toddlers may have infrequent incidental oral exposure resulting from mouthing of paper, there is uncertainty regarding the actual amount ingested and the dye fastness to the paper following oral ingestion. Inhalation and dermal exposures to these dyes from paper products are unlikely due to the physical and chemical properties of these substances as well as the impregnation of the colourants in the paper.

Table 7-1. Summary of estimated exposures to certain Azo Direct Dyes

Substance	Adult - Daily exposure from textiles: personal apparel (dermal, mg/kg-bw per day)	Infant - Daily exposure from textiles: baby sleeper (dermal, mg/kg-bw per day)	Infant - Daily exposure from mouthing of textile objects (oral, mg/kg-bw per day)	Adult - Per event exposure from leather products (dermal, mg/kg-bw)	Infant - Per event exposure from toys of leather material (dermal, mg/kg-bw)
Direct Green 28, Direct Orange 26, Direct Orange 39, Direct Violet 51, Direct Yellow 28	0.0026	0.004	2.7×10^{-5}	N/A	N/A
Direct Red 31, Direct Red 81 triethanolamine salt, Direct Yellow 12, Direct Yellow 50, Direct Black 56, CAS RN 28706-21-0, CAS RN 71033-21-1, CAS RN 83221-56-1, CAS RN 84878-17-1	0.0026	0.004	2.7×10^{-5}	0.0021–0.077	0.04

Abbreviations: kg-bw, kilograms of body weight; N/A, not applicable

In contrast to the direct dyes, reactive dyes generally have very good wet fastness. These dyes are covalently bonded to textile fibres and are not expected to migrate substantially from the material during use and wear (BfR 2007). One study demonstrated the extraction of reactive dyes from three cotton textile samples, using a perspiration (sweat) simulant, at levels up to 158 µg/500 cm^2 (ETAD 1983). However, these extraction experiments, conducted under severe conditions, including high humidity and pressure, are not representative of actual exposure scenarios. If the dyeing and after-treatment procedures have been properly performed, consumer exposure to Reactive Black 5, Reactive Black 158 and Reactive Blue 225 from textile materials is not expected to be significant.

7.1.3 Uncertainties in Human Exposure Assessment

There is uncertainty regarding the exposure characterization from environmental media due to the lack of data. Environmental analysis of sulfonated dyes, including Azo Direct Dyes, is particularly difficult and requires extensive data libraries and a series of chromatographic separation methods (Rehorek and Plum 2007). As such, the absence of literature data may be due to the challenge in quantitative analysis.

In 2010, six Azo Direct Dyes (Direct Black 56, Direct Red 81 triethanolamine salt, CAS RN 28706-21-0, CAS RN 71033-21-1, CAS RN 83221-56-1 and CAS RN 84878-17-1) as well as two Azo Reactive Dyes (Reactive Black 158 and Reactive Blue 225) were identified as being used in Canada based on information submitted by ETAD (personal communication, email from ETAD to Environment Canada, dated 2010; unreferenced); however, the actual uses for these substances were not indicated. It is assumed, based on the information concerning the general uses of direct dyes, that these six Azo Direct Dyes are used as dyes for textiles, leather and paper. Similarly, it was also assumed that Reactive Black 158 and Reactive Blue 225 are used in textile dyeing, based on the literature indicating general uses of reactive dyes.

There is uncertainty regarding the presence in Canada of any of the 61 Azo Direct Dyes and 8 Azo Reactive Dyes in products imported into Canada due to the limited Canadian information available on imported products in general.

There is uncertainty with respect to the estimated exposures from use of textile and leather products dyed using azo dyes. These estimates are based on generic assumptions for concentration of dye in these products and are not specific to Azo Direct Dyes. Consequently, general population exposure estimates are based on conservative assumptions and are likely overestimates. In the absence of dermal absorption data, the dermal absorption of these substances was conservatively assumed to be 100%. Although the dermal uptake of these substances is likely less than 100%, potentially there could be substantial absorption if skin bacteria cleaved azo bonds, thereby releasing aromatic amines, which could then be more readily absorbed.

It is not expected that a given Azo Direct Dye would be present in 100% of consumer products made of textiles in Canada. Therefore based on professional judgment, general population exposures were estimated assuming that there is a 10% probability that an individual Azo Direct Dye is used in dyeing products made of textile in Canada. This adjustment factor, while considered conservative, is similar to the 8% 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 B1 for further explanation.

7.2 Health Effects Assessment

In general, carcinogenicity and genotoxicity are the critical health effects of potential concern for Aromatic Azo and Benzidine-based Substances. Reductive cleavage of the azo bond is considered to be an important metabolic reaction for these substances to exert their toxicity, as it releases some free aromatic amines that are further converted to reactive electrophilic intermediates through metabolic activation (Environment Canada and Health Canada 2013).

Azo Direct Dyes and Azo Reactive Dyes are large water-soluble substances with sulfonic acid substituent(s) (SO^{3-}) and/or other ionized functional group(s) at one or more positions (refer to Section 3). Such characteristics limit the penetration of the intact dye molecule through skin or gastrointestinal tract, but make them more susceptible to skin or gut bacteria-mediated azo bond reduction as observed in the *in vitro* tests for some of the Azo Direct Dyes and Azo Reactive Dyes in this assessment (refer to Section 7.2.1 and 7.2.2). *In vivo* studies on Reactive Black 5 (Section 7.2.2) and other sulfonated azo dyes, such as Amaranth and New Coccine, also showed that these dyes were reduced following oral administration and the released aromatic amines were absorbed into systemic circulation (Environment Canada and Health Canada 2014a). Bioabsorption of the relatively smaller aromatic amine metabolites is expected to dominate over absorption of the intact parent dyes. Azo bond reductive cleavage is thus considered to be the main bioactivation pathway for the Azo Direct Dyes and Azo Reactive Dyes.

Only limited health effects data have been identified for the 69 substances (61 Azo Direct Dyes and 8 Reactive Dyes) (Appendix C). The focus of this assessment is on the 21 substances (18 Azo Direct Dyes and 3 Azo Reactive Dyes) for which potential exposure has been identified (refer to Section 7.1). As available data for these 21 substances are limited, health information on their component aromatic amines was considered to inform the hazard potential for these substances. Similarly, data on other structurally similar substances outside these subgroups were also considered.

Direct Orange 26 and CAS RN 83221-56-1 are considered to be toxicologically equivalent, as the two substances contain the same organic moiety and sodium ion. Similarly, empirical data on Direct Yellow 11 and Direct Red 81 are considered applicable to their salts (lithium and triethanolamine salts, respectively). The potential health effects of lithium ion and triethanolamine were not considered relevant to the health effects assessment of these dyes because these small, highly water-soluble molecules will be washed off during dyeing and washing processes, and therefore are not present in the finshed products (personal communication, email from Dr. H. Freeman, North Carolina State University, dated 2013; unreferenced).

In the following sections, the azo bond reductive cleavage potential and critical health effects, including carcinogenicity, genotoxicity and other health effects, are evaluated for the Azo Direct Dyes and Azo Reactive Dyes, respectively.

7.2.1 Azo Direct Dyes

7.2.1.1 The 18 Azo Direct Dyes with Potential Exposure

Direct Black 56, Direct Blue 71, Direct Green 28, Direct Orange 26, Direct Orange 39, Direct Red 31, Direct Red 81 triethanolamine salt, Direct Violet 51, Direct Yellow 11, Direct Yellow 11 lithium salt, Direct Yellow 12, Direct Yellow 28, Direct Yellow 34, Direct Yellow 50, CAS RN 28706-21-0, CAS RN 71033-21-1, CAS RN 83221-56-1 and CAS

RN 84878-17-1 have been identified as being used in products available to consumers in the Canadian marketplace and therefore general population exposure to these substances may occur (refer to Section 7.1).

7.2.1.1.1 Azo Bond Cleavage Potential

Azo bond reductive cleavage was tested *in vitro* for some of the Azo Direct Dyes under anaerobic conditions with human fecal bacterial cultures and/or under aerobic conditions with the culture of human skin bacteria, *Staphylococcus epidermidis* or *Micrococcus luteus*, for 24 hours (BRI 2012, 2013a, b). The results are summarised in Table 7-2. Overall, all tested Azo Direct Dyes underwent azo bond reductive cleavage under both conditions, but to varying degrees of completion.

Table 7-2. Summary of azo reductive cleavage testing results

Azo Direct Dye	Anaerobic[a] with Human fecal bacteria culture	Aerobic[b] with *Staphylococcus epidermidis*	Aerobic with *Micrococcus luteus*
Direct Blue 71	Fast	NT	NT
Direct Green 28	Moderate	Fast	Fast
Direct Orange 26	Moderate	Moderate	Moderate
Direct Orange 39	Slow	Slow	Slow
Direct Red 31	Fast	NT	NT
Direct Yellow 11	Slow	Moderate	Moderate
Direct Yellow 12	Moderate	Fast	Fast
Direct Yellow 50	Fast	NT	NT
Direct Violet 51	Fast	Negative[c]	Positive[c]

Abbreviation: NT, not tested

[a] Under anaerobic conditions, the relative azo bond reduction rates were ranked as “fast” (the azo bond reduction was completed over 24 hours), “moderate” (gradually occurred over 24 hours) or “slow” (only minimal azo bond reduction was observed over 24 hours).

[b] Under aerobic testing conditions, the relative azo bond reduction rates were ranked as“fast” (the azo bond reduction was completed within 6 hours), “moderate” (the azo bond reduction was completed over 24 hours) or “slow” (the azo bond reduction occurred slowly and was not completed over 24 hours) (BRI 2013a, b).

[c] This substance was tested in a different experiment and its azo bond reduction rate was not compared with the other substances (BRI 2012).

In addition, other studies also reported biodegradation of Direct Blue 71 (Abadulla et al. 2000; Tauber et al. 2008; Hsu et al. 2012), Direct Orange 39 (Jadhav et al. 2010) and Direct Violet 51 (Enayatzamir et al. 2009; Corso et al. 2012) by bacteria *Pseudomonas* or fungi.

Based on the available information, it is considered that all 18 Azo Direct Dyes have the potential to be severed at the azo bond following contact with either skin or gut microflora.

7.2.1.1.2 Carcinogenicity and Genotoxicity

The carcinogenic and genotoxic potential of the 18 Azo Direct Dyes were evaluated based on available empirical data on the dyes and their postulated azo bond reductive cleavage products (i.e., aromatic amines) and structurally similar substances within and/or outside this subgroup.

No cancer bioassay data for the 18 Azo Direct Dyes were identified. Limited genotoxicity data have been identified for eight of the Azo Direct Dyes as summarized in Table 7-3.

Table 7-3. Summary of available genotoxicity data for Azo Direct Dyes

Azo Direct Dye	***In vivo* MN**	***In vivo* DL**	***In vitro* reAmes**	***In vitro* stdAmes**	***In vitro* UDS**	***In vitro* CA**	***In vitro* ML**	***In vitro* SOS**
Direct Blue 71	–	–	P	P	–	–	–	–
Direct Green 28	–	–	N	N	–	–	–	–
Direct Orange 39	–	–	–	N	–	N	N	N
Direct Red 31	–	–	–	–	–	–	–	N
Direct Red 81[a]	P	N	–	N	N	–	–	–
Direct Yellow 11	–	–	–	N	–	–	–	–
Direct Yellow 12	P	–	P	N	–	–	–	N
Direct Yellow 50	–	–	P	P	–	–	–	–

Abbreviations: –, no data identified; CA, chromosomal aberration in mammalian cells; DL, dominant lethal mutation; ML, gene mutation test in mouse lymphoma; MN, micronucleus induction; N, negative; P, positive; reAmes, reductive Ames test; SOS, SOS response/umu tests in bacteria; stdAmes, standard Ames test; UDS, unscheduled DNA synthesis in mammalian cells

[a] Data for Direct Red 81 are presented to inform the genetoxicity potential of Direct Red 81 triethanolamine salt.

In vivo, Direct Red 81 (data applicable to Direct Red 81 triethanolamine salt) and Direct Yellow 12 significantly induced micronucleus formation in mouse bone marrow following intraperitoneal injection; the two substances also caused mouse bone marrow erythrocyte cytotoxicity (Przybojewska et al. 1988, 1989). Direct Red 81 did not induce dominant lethal mutations in male mice following intraperitoneal injection (Przybojewska et al. 1988).

In vitro, Ames tests were conducted under both standard and reductive conditions (with and without flavin mononucleotide [FMN]) for four Azo Direct Dyes in *Salmonella typhimurium* strains TA98 and TA100 with metabolic activation (ILS 2011). Direct Blue 71 was positive in both strains with and without FMN; Direct Yellow 12 was positive in both strains with FMN, but negative without FMN; Direct Yellow 50 was positive in TA98 with and without FMN, positive in TA100 with FMN and negative in TA100 without FMN; and Direct Green 28 was negative in both strains with and without FMN. In the standard Ames tests, Direct Yellow 11 was negative in strains TA98, TA100, TA1535 and TA1537, and Direct Orange 39 was negative in strains TA97, TA98, TA100, TA102, TA1535 and TA1537, with and without metabolic activation (Zeiger et al. 1987; ETAD 1988; REACH 2013a). Direct Red 81 and Direct Yellow 12 were negative in strains

TA97, TA98, TA1535, TA1537 and TA1538 with and without metabolic activation (Przybojewska et al. 1988). Direct Orange 39 was negative in chromosomal aberration tests in V79 cells and gene mutation tests in mouse lymphoma cells, with and without metabolic activation (REACH 2013a). *In vitro*, Direct Red 81 did not induce DNA damage and repair (unscheduled DNA synthesis) in primary rat hepatocytes (Palus et al. 1995). Additionally, Direct Orange 39, Direct Red 31 and Direct Yellow 12 did not induce SOS response (umu test) in bacteria (Nakamura et al. 1993).

Overall, available genotoxicity data indicate that of the eight Azo Direct Dyes, only Direct Yellow 12 tested positive *in vivo* and *in vitro*; although Direct Red 81 caused some clastogenicity and cytotoxicity *in vivo*, it did not exhibit mutagenicity *in vivo* or *in vitro*. Direct Blue 71 and Direct Yellow 50 were mutagenic in bacteria in one study.

7.2.1.1.2.1 Health Information on Structurally Similar Substances

Due to the lack of carcinogenicity data on the Azo Direct Dyes in this assessment, searches for additional structurally similar substances outside this subgroup with cancer bioassay data were conducted using the OECD QSAR Toolbox (2013), Health Canada internal databases, ChemIDplus (1993–) and toxicological literature (Toxnet and SciFinder). The search criteria included dye application class, structural features (absence of benzidine moiety, presence of benzene and/or naphthalene ring(s), number of azo bond(s) and presence of sulfonic acid substituent(s)), physical and chemical properties (molecular size and solubility) and mode of action (potential to release the same aromatic amines via azo bond reductive cleavage). No cancer bioassay data for azo direct dyes were identified. However, three azo acid dyes (Amaranth, New Coccine and Acid Red 1) were identified. The health effects of Amaranth and New Coccine have been assessed along with Certain Azo Acid Dyes in a separate Screening Assessment under the Chemicals Management Plan (Environment Canada and Health Canada 2014a). The health effects of Acid Red 1 have been reviewed by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) (WHO 1981). None of these three substances exhibited evidence of carcinogenicity in experimental animals.

7.2.1.1.2.2 Postulated Azo Bond Reductive Cleavage Products

Available data indicate that sulfonated aromatic amines generally have low carcinogenic and genotoxic potential owing to their high electronegativity and water solubility (Marchisio et al. 1976; Lin and Solodar 1988; Jung et al. 1992; OECD QSAR Toolbox 2013). As not all of the component aromatic amines of the 18 Azo Direct Dyes contain a sulfonic acid moeity, the carcinogenic and genotoxic potential of the postulated azo bond reductive cleavage products that are unsulfonated were considered to inform the health effects of their parent dyes.

Empirical cancer and genotoxicity bioassay data have been identified for some of the postulated azo bond reductive cleavage products of the 18 Azo Direct Dyes. These data as well as the sulfonation status of these postulated products (i.e., aromatic amines) are presented and summarized in Appendix C.

Among the six aromatic amines (4-Nitroaniline, p-Phenylenediamine, Aniline, 4,4′-Diamino-2,2′-stilbenedisulfonic acid, Amido-G-Acid, Mesalamine) that have cancer bioassay data available, aniline is the only substance that has exihibited clear carcinogenic evidence in male rats. Aniline-based dyes, i.e., Direct Black 56, Direct Orange 26, Direct Red 31 and CAS RN 83221-56-1, may have similar health effects if a substantial amount of aniline is released by azo cleavage. However, it is unknown to what extent the azo bond reduction of these dyes occurs *in vivo* to release aniline. Direct Green 28, Direct Orange 39, Direct Red 81 (equivalent to Direct Red 81 triethanolamine salt in the finished products), Direct Yellow 11 and its lithium salt, Direct Yellow 12, Direct Yellow 28 and CAS RN 71033-21-1 are considered to have low carcinogenic potential based on information on the postulated azo bond cleavage products (lack of carcinogenicity evidence in experimental animal studies and/or the presence of sulfonic acid substituents). The carcinogenic potential of Direct Blue 71, Direct Violet 51, Direct Yellow 34, Direct Yellow 50, CAS RN 28706-21-0 and CAS RN 84878-17-1 can not be conclusively determined owing to the lack of empirical data for their unsulfonated component aromatic amine(s).

With respect to genotoxicity, available data indicate that aniline and *p*-phenetidine exhibited some genotoxic potential *in vivo* and *in vitro*, suggesting that Direct Black 56, Direct Orange 26, Direct Red 31, Direct Yellow 12 and CAS RN 83221-56-1 may also have similar genotoxic effects. Again, it is unknown to what extents aniline and *p*-phenetidine are released from these dyes *in vivo*. For Direct Yellow 12, the results are consistent between the parent dye (Direct Yellow 12) and its metabolite (*p*-phenetidine). Although *p*-phenylenediamine showed some genotoxic effects *in vitro*, the *in vivo* test results were negative. 4-nitroaniline is considered to have weak genotoxic potential. Mesalamine is not genotoxic.

Based on the available information, Direct Black 56, Direct Orange 26, Direct Red 31, Direct Yellow 12 and CAS RN 83221-56-1 are considered to have genotoxic potential. Direct Green 28, Direct Orange 39, Direct Red 81 (equivalent to Direct Red 81 triethanolamine salt in the finished products), Direct Yellow 11 and its lithium salt, Direct Yellow 28 and CAS RN 71033-21-1 are considered to have low genotoxic potential based on information on the postulated azo bond cleavage products (lack of genotoxicity evidence in experimental studies and/or the presence of sulfonic acid substituents). The genotoxic potential of Direct Blue 71, Direct Violet 51, Direct Yellow 34, Direct Yellow 50, CAS RN 28706-21-0 and CAS RN 84878-17-1 can not be conclusively determined owing to the lack of empirical data for their unsulfonated component aromatic amine(s).

7.2.1.1.3 Other Health Effects

7.2.1.1.3.1 Repeated-Dose Toxicity

Among the 18 dyes, repeated-dose toxicity data were identified only for Direct Orange 39 in a REACH dossier (one study) (REACH 2013a). Searches for structurally similar susbtances outside this subgroup that have repeated-dose toxicity data available was conducted using the same approach as described above. Six additional substances were identified: Direct Black 19 was identified in the OECD QSAR Toolbox (2013) and in the public domain (REACH 2013b); Acid Black 210 was identified as an analogue of Direct Black 19 (REACH 2013b); an azo direct dye, NSN, was identified in an internal database [Health Canada]; Amaranth, New Coccine and Acid Red 1 were identified in ChemIDplus (1993-). Data for Amaranth, New Coccine and Acid Red 1 were available on potential health effects following subchronic and chronic exposure, while Direct Orange 39, Direct Black 19 and Acid Black 210 only have toxicological data for short-term exposure. Among the six additional substances identified, health effects information for Acid Red 1 is of particular significance as this dye releases aniline through azo bond reductive cleavage and elicits health effects similar to aniline. Aniline is the only postulated azo bond reductive product potentially released from the 18 Azo Direct Dyes that has an indication of carcinogenicity (see above). Based on available data in the OECD QSAR Toolbox (2013), aniline is also one of the most potent non-benzidine aromatic amines in terms of chronic toxicity. Effect levels for Acid Red 1 thus represent the lower bound of the range of potential health effect levels for the 18 Azo Direct Dyes. However, owing to structural diversity, the health information on the six additional substances does not necessarily encompass the general health effects of the 18 Azo Direct Dyes; rather it provides a range of no observed adverse effect levels (NOAELs) below which health effects are not expected.

Tables 7-4 and 7-5 present available critical effect levels for these substances via oral and dermal routes of exposure, respectively. Health effects data for Direct Orange 39, Direct Black 19, Acid Black 210 and Acid Red 1 are summarized below. Amaranth and New Coccine were evaluated along with Certain Azo Acid Dyes in a separate Screening Assessmnet, in which detailed summaries of their health effects were presented (Environment Canada and Health Canada 2014a). A range of NOAELs, 26–300 mg/kg body weight (kg-bw) per day, was identified from oral repeated-dose toxicity studies for Direct Orange 39 and the six additional substances. Data from the dermal route were limited; only a single test dose level was used in each of the available short-term and chronic dermal toxicity studies.

Table 7-4. Overview of relevant critical oral health effect levels

Test substance (reference)	Short-term toxicity (mg/kg-bw per day)	Subchronic toxicity (mg/kg-bw per day)	Chronic toxicity (mg/kg-bw per day)	Reproductive toxicity (mg/kg-bw per day)	Developmental toxicity (mg/kg-bw per day)
Direct Orange 39 (REACH	NOEL = 30 NOAEL[1] = 300	No data	No data	NOEL = 750 No effects	NOEL = 750 No effects (42-day study in

Test substance (reference)	Short-term toxicity (mg/kg-bw per day)	Subchronic toxicity (mg/kg-bw per day)	Chronic toxicity (mg/kg-bw per day)	Reproductive toxicity (mg/kg-bw per day)	Developmental toxicity (mg/kg-bw per day)
2013a)	LOAEL = 750 Hematological effects, reductions in body weight and feed intake, increases in liver and kidney weights (42-day study in rats via gavage)			(42-day study in rats via gavage)	rats via gavage)
Direct Black 19 (REACH 2013b)	NOAEL[1] = 80 LOAEL = 320 Lesions in myocardium, larynx and skeletal muscle and pigment accumulation in these organs (42-day study in rats via gavage)	No data	No data	NOAEL = 80 LOAEL = 320 Decrease in the number of females bearing live pups and mean number of pups per litter (42-day study in rats via gavage)	NOAEL = 80 LOAEL = 320 Pup mortality, increased preimplantation loss, decreased mean litter weight and mean pup weight at postnatal day 4 (42-day study in rats via gavage)
Acid Black 210 (REACH 2013b)	NOAEL[1] = 150 LOAEL = 450 Clinical chemistry and hematological parameter changes, in	No data	No data	NOEL = 150 LOAEL = 450 Slightly slower sperm motility,	NOEL = 150 LOAEL = 450 Decreased total number of live pups and uterine implantation (42-day study

Test substance (reference)	**Short-term toxicity (mg/kg-bw per day)**	**Subchronic toxicity (mg/kg-bw per day)**	**Chronic toxicity (mg/kg-bw per day)**	**Reproductive toxicity (mg/kg-bw per day)**	**Developmental toxicity (mg/kg-bw per day)**
	splenic hematopoiesis, lymphoid enlargement, multiple organ weights and colour changes (42-day study in rats via gavage)			slightly decreased number of females achieving pregnancy and number of corpora lutea (42-day study in rats via gavage)	in rats via gavage)
Amaranth (Environment Canada and Health Canada 2014a)	No data [see Developmental toxicity data]	LOAEL = 80 Kidney calcification (90-day study in rats via diet)	LOAEL[1] = 50 Kidney calcification (2-year study in rats via diet)	NOAEL ≥ 1250 No effects (two- and three-generation studies in rats via diet)	NOAEL ≥ 15 No effects (multiple studies in rats, rabbits and dogs via diet or gavage)
New Coccine (Environment Canada and Health Canada 2014a)	No data [see Developmental toxicity data]	No data	NOAEL[1] = 65 LOAEL = 325 Glomerulonephrosis and mild anemia (80-week study in mice via diet)	NOAEL = 1250 No effects (three-generation study in rats via diet)	NOAEL > 100 No effects (three-generation study in rats via diet and multiple developmental toxicity studies in mice and rats via diet or gavage)
Acid Red 1 (WHO 1981)	[Multiple studies available, blood and spleen effects	[Multiple studies available, blood and spleen	NOAEL[1] = 26–43 LOAEL = 130–215 Splenic	NOAEL = 187.5 No effects (two-generation	NOAEL = 187.5 No effects (exposure during

Test substance (reference)	Short-term toxicity (mg/kg-bw per day)	Subchronic toxicity (mg/kg-bw per day)	Chronic toxicity (mg/kg-bw per day)	Reproductive toxicity (mg/kg-bw per day)	Developmental toxicity (mg/kg-bw per day)
	observed]	effects observed]	erythropoiesis, hemosiderin in spleen and kidney (80-week study in mice via diet) NOAEL = 8 LOAEL = 32 Splenic erythropoiesis, hemosiderin in liver, spleen and kidney, splenomegaly (2-year study in rats via diet)	study in rats via diet)	gestation days 0–19 in rats via diet)

Abbreviations: kg-bw, kilogram of body weight; LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level; NOEL, no-observed-effect level

[1] Critical health effect level of the substance.

Table 7-5. Overview of relevant critical dermal health effect levels

Test substance (reference)	Short-term toxicity (mg/kg-bw per day)	Subchronic toxicity (mg/kg-bw per day)	Chronic toxicity (mg/kg-bw per day)	Reproductive toxicity (mg/kg-bw per day)	Developmental toxicity (mg/kg-bw per day)
NSN (Health Canada internal database)	LOAEL > 500 Local effects (28-day study in rats, single dose level)	No data	No data	No data	No data
Amaranth (Environment Canada and Health Canada 2013c)	No data	No data	NOEL = 5 No effects (single dose level, once a	No data	No data

Test substance (reference)	Short-term toxicity (mg/kg-bw per day)	Subchronic toxicity (mg/kg-bw per day)	Chronic toxicity (mg/kg-bw per day)	Reproductive toxicity (mg/kg-bw per day)	Developmental toxicity (mg/kg-bw per day)
			week dermal application in mice for 19.5 months)		

Abbreviations: kg-bw, kilogram of body weight; LOAEL, lowest-observed-adverse-effect level; NOEL, no-observed-effect level

Direct Orange 39

Toxicity data for Direct Orange 39 were reported in a REACH dossier (REACH 2013a). In a combined repeated-dose toxicity study with the reproduction/developmental toxicity screening test conducted according to OECD Test Guideline 422, Wistar rats were administered Direct Orange 39 at a dose level of 0, 30, 300 or 750 mg/kg-bw per day by gavage for 15 days prior to mating until 4 days postpartum for females or for a total of 42 days for males. The NOAEL of this study was 300 mg/kg-bw per day and the LOAEL was 750 mg/kg-bw per day based on systemic toxicity, including reduction in hemoglobin, erythrocyte and hematocrit counts with increased mean red blood cell volume and mean red blood cell hemoglobin in the treated males, reduction of body weight gain (males), body weight loss (females), reduced feed intake (both sexes), increased water intake (both sexes), and increased liver and kidney weights (both sexes), along with maternal toxicity-caused reproductive and developmental effects, such as slightly increased gestation lengths, smaller litter sizes, lower body weights of offspring, lower numbers of corpora lutea and implantation sites and higher pre- and post-implantation losses.

Direct Black 19 (Additional substance, CAS RN 6428-31-5)

Direct Black 19 was the only azo direct dye outside this subgroup that has available empirical data in the open literature, which were reported in another REACH dossier (REACH 2013b). In a combined repeated-dose toxicity study with the reproduction/developmental toxicity screening test conducted according to OECD Test Guideline 422, Wistar rats were administered Direct Black 19 at a dose level of 0, 20, 80 or 320 mg/kg-bw per day by gavage for 14 days prior to mating until 4 days postpartum for females or for a total of 42 days for males. Additional male and female rats in the satellite group were administered the highest dose level for 42 days and observed for another 2 weeks. The NOAEL for this study was 80 mg/kg-bw per day and the LOAEL was 320 mg/kg-bw per day for systemic, reproductive and developmental toxicity, based on fibrosis and pigment accumulation in myocardium, larynx muscle and skeletal muscle (more severe in females than in males) and atrophic changes in thymus of females present after 2 weeks of recovery, as well as decreased number of live pups, increased preimplantation losses, decreased mean litter weights and decreased mean pup weights, which were attributed to maternal toxicity. Accumulation of pigment in other tissues (brain, spleen, thyroid gland and kidneys), reduction in body weight gains, some clinical chemistry and urinary measurements, hematological changes (platelet and leucocyte counts and prothrombin and partial thromboplastin times) and organ (thymus, heart and spleen) weight changes were also observed (REACH 2013b).

Acid Black 210 (Additional substance)

The REACH dossier for Direct Black 19 also includes data for Acid Black 210 as supporting health information (REACH 2013b). In a combined repeated-dose toxicity study with the reproduction/developmental toxicity screening test conducted according to OECD Test Guideline 422, Wistar rats were administered Acid Black 210 at a dose level of 50, 150 or 450 mg/kg-bw per day by gavage for 14 days prior to mating until day 4 postpartum for females or for a total of 42 days for males. Additional male and female rats in the satellite group were administered the highest dose level for 42 days and observed for an additional 2 weeks. The NOAEL for systemic effects was established to be 150 mg/kg-bw per day, based on mild spleen enlargement and colour changes (both sexes), mild spenic hematopoiesis (males), enlarged lymphoid sheaths (males) and glaringly yellow colour and presence of leucocytes in the urine (males); the LOAEL was considered to be 450 mg/kg-bw per day, based on significant clinical chemistry and hematological parameter changes, multiple organ weights and colour changes, extramedullary hematopoiesis in the spleen and enlargement of the lymphoid. The no-observed-effect level (NOEL) for reproductive and developmental toxicity was established to be 150 mg/kg-bw per day and the LOAEL to be 450 mg/kg-bw per day, based on decreased total number of live pups and average number of pups per litter, slightly slower sperm motility, slightly decreased number of females achieving pregnancy and decreased number of corpora lutea and uterine implantations (REACH 2013b).

Acid Red 1 (Additional substance, CAS RN 3734-67-6)

The molecular structure of Acid Red 1 contains 1-hydroxyl, 2-phenylazo and 3-naphthalenesulfonic acid moieties, similar to the chemical structures of Direct Orange 26, Direct Red 31 and CAS RN 83221-56-1. This suggests a comparable potential and rate of azo bond reductive cleavage to release aniline, leading to a similar health outcome for these substances. In the animal studies (including short-term, subchronic, chronic, two-generation reproductive and developmental toxicity studies) reviewed by JECFA (WHO 1981), following oral administration (gavage or diet) of Acid Red 1 in rats and rabbits, aniline and aniline metabolites (about half of the administered dose) were detected in the urine of treated animals. Typical aniline-induced health effects, such as methemoglobinemia, Heinz bodies, reticulocytosis, decreased hemoglobin, packed cell volume and blood cell counts and increased spleen weight and erythropoiesis in the liver, spleen and bone marrow, were observed in orally administered rats and mice in repeated-dose toxicity studies. Evidence of carcinogenicity was not observed in the treated rodents up to the highest doses tested (mice, up to 0.625% in diet [812.5 mg/kg-bw per day] for 80 weeks; rats, up to 0.16% in diet [80 mg/kg-bw per day] for 2 years). JECFA considered the NOAEL for mouse to be 0.025% in the diet for 80 weeks (equivalent to 26–43 mg/kg-bw per day) and the NOAEL for rat to be 0.016% in the diet for 2 years (equivalent to 8 mg/kg-bw per day; LOAEL = 32 mg/kg-bw per day based on splenic enlargement, hemosiderin and necrosis) (WHO 1981).

Considering aniline was the proximate toxicant for Acid Red 1, available data for aniline (Health Canada 2011a) were considered in the determination of the point of departure (POD) for oral repeated-dose toxicity of Acid Red 1. In oral chronic studies, the lowest LOAEL for aniline was 7.2 mg/kg-bw per day in treated CD-F male rats, whereas in treated CD-F female rats, the NOAEL and LOAEL were 7.2 and 21.6 mg/kg-bw per day, respectively. In aniline-treated male Wistar rats, male and female Fischer 344 rats and B6C3F1 mice, the reported LOAELs were all at higher dose levels. The NOAELs observed in Acid Red 1–treated rats and mice were 8 and 26 mg/kg-bw per day, respectively, equivalent to 1.5–4.8 mg/kg-bw per day of aniline assuming complete release of anilinefrom Acid Red 1. As these calculated NOAELs are all below the lowest LOAEL for aniline (7.2 mg/kg-bw per day), the higher NOAEL of 26 mg/kg-bw per day was considered to be the POD for Acid Red 1.

7.2.1.1.3.2 Acute Toxicity

Oral acute toxicity data reported in REACH dossiers (REACH 2013a, b) for Direct Orange 39 (purity 27%) and Direct Black 19 (additional substance) indicated that no deaths or adverse effects were observed in Direct Orange 39– or Direct Black 19–treated rats at 2000 mg/kg-bw via gavage. Dermal acute toxicity data reported in REACH (2013a) for Direct Orange 39 also indicated that no animal deaths or adverse effects were observed at 2000 mg/kg-bw in rats and rabbits. In the absence of acute toxicity data for other Azo Direct Dyes, these substances are considered to have generally low oral and dermal acute toxicity based on the available data on Direct Orange 39 and Direct Black 19 as well as data on Certain Azo Acid Dyes (Environment Canada and Health Canada 2014a).

7.2.1.1.3.3 Sensitization and Irritation

Some Azo Direct Dyes were notified by stakeholders in the EU to be classified for their sensitization or irritation: Direct Orange 39 and Direct Yellow 11 to be in Skin Sensitization category 1; Direct Blue 71, Direct Orange 26, Direct Orange 39, Direct Red 81, Direct Violet 51, Direct Yellow 12, Direct Yellow 28, CAS RN 83221-56-1 and CAS RN 84878-17-1 to be in Eye Irritation category 2; Direct Orange 26, Direct Red 81 and CAS RN 83221-56-1 to be in Skin Irritation category 2; and Direct Orange 39 to be in Specific target organ toxicity single exposure category 3 (ECHA 2013).

Some sensitization and irritation testing data for Direct Orange 39 were reported in the REACH (2013a) dossier. Inconsistent sensitization test results in guinea pigs and negative results in the human patch test were observed. Direct Orange 39 induced moderate skin irritation and slight eye irritation in rabbits.

7.2.1.2 The Remaining Azo Direct Dyes

No empirical health effects data were identified for the remaining 43 Azo Direct Dyes for which potential exposure has not been identified (Appendix C). Among these substances, seven Azo Direct Dyes can potentially release EU22 aromatic amines via azo bond reductive cleavage: Direct Red 62, CAS RN 72749-87-2, CAS RN 83232-30-8 and CAS RN 83232-32-0 can release *o*-toluidine (CAS RN 95-53-4); Direct Red 26 and CAS 72749-88-3 can release *o*-anisidine (CAS RN 90-04-0); and CAS RN 84878-16-0 can release 4,4′-thiobisbenzenamine (CAS RN 139-65-1).

7.2.2 Azo Reactive Dyes

7.2.2.1 Three Azo Reactive Dyes with Potential Exposure

Reactive Black 5, Reactive Black 158 and Reactive Blue 225 have been identified as being used in products available to consumers in the Canadian marketplace and therefore general population exposure to these substances may occur (refer to Section 7.1).

7.2.2.1.1 Azo Bond Cleavage Potential

Azo bond reductive cleavage of Reactive Black 5 was tested under anaerobic and aerobic conditions (BRI 2013a, b). Reactive Black 5 was cleaved under both conditions: gradual and incomplete azo bond reduction occurred over 24 hours with human fecal bacterial cultures and complete azo bond reduction within 1 hour occurred with the culture of human skin bacteria, *Staphylococcus epidermidis* or *Micrococcus luteus* (BRI 2013a, b). In addition, biodegradation by fungi or fungal enzymes *in vitro* has been reported for Reactive Black 5 and other azo reactive dyes (i.e., Reactive Black 19 and Reactive Black 221) (Abadulla et al. 2000; Tauber et al. 2008).

Data from rat *in vivo* studies in which a single Reactive Black 5 dose was administered by gavage showed that Reactive Black 5 was absorbed and excreted mainly in feces and to a lesser extent in urine. The main metabolite identified resulted from azo bond reductive cleavage (REACH dossier 2013c).

Based on read-across from Reactive Black 5, Reactive Black 158 and Reactive Blue 225 are also considered to have potential to undergo azo bond reductive cleavage since no empirical data for these two Azo Reactive Dyes were identified.

7.2.2.1.2 Carcinogenicity and Genotoxicity

Adequate cancer bioassay data for the three Azo Reactive Dyes were not identified. One older study examined the carcinogenicity of Reactive Black 5 in rats and mice via oral administration, however, no concurrent control animals were used; hence, an evaluation of dose-related effects was not possible (REACH 2013c).

In vivo and *in vitro* genotoxicity data for Reactive Black 5 were reported in the REACH dossier (2013c). Reactive Black 5 did not induce chromosomal aberration in Chinese hamsters or micronucleus formation in mice following single oral gavage administration. It did not induce gene mutation in bacteria (Ames test) with or without metabolic activation. In addition, a postulated azo bond reductive cleavage product of Reactive Black 5 and Reactive Blue 225, 4-((2-sulfatoethyl)sulfonyl)aniline (CAS RN 2494-89-5), did not induce chromosomal aberration in mammalian cells or gene mutation in bacteria (Ames test) *in vitro* with or without metabolic activation (REACH 2013d). Although genotoxicity testing data for Reactive Black 158 and Reactive Blue 225 have not been identified, all of their component aromatic amines are sulfonated (Appendix C) and are therefore expected to have low genotoxic potential.

Overall, the three Azo Reactive Dyes are considered to have low carcinogenic and genotoxic potential.

7.2.2.1.3 Other Health Effects

Repeated-Dose Toxicity

Repeated-dose toxicity data were identified only for Reactive Black 5. Two oral studies for Reactive Black 5 were reported in the REACH dossier (2013c). In the short-term study (21 days), albino rats were given Reactive Black 5 by gavage at 500 mg/kg-bw per day, 5 days/week, for 3 weeks (14 treatments in total), followed by an additional 3 days of observation. No treatment-related health effects were observed as evaluated by clinical signs, body weights, urinalysis, hematological analysis and macroscopic and microscopic examination. In the developmental toxicity study, pregnant Wistar rats were administered Reactive Black 5 at 1000 mg/kg-bw per day by gavage during gestation days 7–16. No treatment-related health effects on the dams or developmental effects on the fetuses were observed (black discoloration of feces and blue discoloration of urine due to excretion of the dye were observed in dams). Health information provided by the two studies is limited as only a single dose level was tested in each.

Acute Toxicity

Acute toxicity data for Reactive Black 5 were reported in the REACH dossier (2013c). Reactive Black 5 did not cause animal deaths or adverse effects in acute oral toxicity tests in rats at 5000 mg/kg-bw via gavage (slight bluish discoloration of the upper layers of the skin (cutis and subcutis) and purple discoloured kidneys were observed).

Sensitization and Irritation

Reactive Blue 225 has been classified as a Skin Sensitization Category 1 substance (ECHA 2013). Reactive Black 5 was notified by stakeholders in the EU to be classified in Respiratory Sensitization category 1, Eye Damage category 1, Skin Irritation category 2, Eye Irritation category 2 and Specific target organ toxicity single exposure category 3 (ECHA 2013).

In addition, it was reported that Reactive Black 5 induced asthma, rhinitis and positive skin patch tests in some patients (Estlander 1988; Nilsson et al. 1993; Pérez-Crespo et al. 2009; REACH 2013c). Most of the patients had occupational exposures to reactive dyes.

In experimental animals, Reactive Black 5 did not cause skin irritation or eye irritation in rabbits or skin sensitization in guinea pigs. Reactive Black 5 did not cause respiratory sensitization in guinea pigs, although immunoglobulin G and allergic antibodies were induced in some of the studies without altering general pulmonary function of the guinea pigs (REACH 2013c).

7.2.2.2 The Remaining Five Azo Reactive Dyes

Empirical health effects data for the remaining five Azo Reactive Dyes have not been identified (Appendix C). Since these Azo Reactive Dyes and their component aromatic amines are all sulfonated, they are considered to have low carcinogenic and genotoxic potential.

7.2.3 Uncertainties in Human Health Effects Assessment

Azo Direct Dyes

Although evidence for azo bond reductive cleavage is available for some of the Azo Direct Dyes, there is uncertainty with respect to the rate and extent of azo bond reduction and the stability of the azo bond reductive cleavage products, which would influence their associated health effects. For example, Acid Red 1 was not carcinogenic in experimental animals, although one of its postulated azo bond reductive cleavage products is aniline, which has induced carcinogenic effects in male rats. There is also uncertainty with respect to the azo bond reductive cleavage products whose health effects have not been investigated or well characterized.

There is high uncertainty with respect to the actual health effects of the Azo Direct Dyes as most of them have not been tested and given the structural diversity, the identified additional substances can not be viewed as toxicologically equivalent to these dyes.

There is also uncertainty with respect to potential health effects of trace level of impurities or minor components of unknown or variable compositions in the dyes

Azo Reactive Dyes

For the Azo Reactive Dyes, empirical health effects data were identified only for Reactive Black 5. There is uncertainty in applying read-across approach for azo bond reductive cleavage for the substances that do not have empirical data. Also, there is high uncertainty with respect to the actual health effects of those no data substances. However, as these dyes and their postulated azo bond reductive cleavage products (aromatic amines) are all sulfonated, they are generally considered to be of low hazard potential.

7.3 Characterization of Risk to Human Health

7.3.1 Azo Direct Dyes

For the general population of Canada, environmental media are not considered to be a significant source of exposure to the Azo Direct Dyes; therefore risk to human health from these sources is considered to be low.

Fourteen of the Azo Direct Dyes (Direct Black 56, Direct Green 28, Direct Orange 26, Direct Orange 39, Direct Red 31, Direct Red 81 triethanolamine salt, Direct Violet 51, Direct Yellow 12, Direct Yellow 28, Direct Yellow 50, CAS RN 28706-21-0, CAS RN 71033-21-1, CAS RN 83221-56-1 and CAS RN 84878-17-1) were identified to be used as dyes in textiles. Daily exposures to the 14 dyes as a result of direct and prolonged dermal contact were estimated to be 0.0026–0.004 mg/kg-bw per day. Daily exposure to these dyes as a result of mouthing of textiles by infants was estimated to be 2.7×10^{-5} mg/kg-bw per day.

A range of NOAELs of 26–300 mg/kg-bw per day based on oral repeated-dose toxicity studies for Direct Orange 39 and six additional related substances was used as a POD to characterize the risk to human health from exposure to the 14 dyes via contact with textiles. Oral data were used in the absence of adequate dermal toxicological data. Comparison of the estimates for dermal exposure to the 14 dyes via textile clothing with the POD results in margins of exposure (MOEs) ranging from 6500 to 115 000. Comparison of the estimates for oral exposure to the 14 dyes via textile mouthing with the POD results in MOEs of ≥ 963 000. These MOEs are considered adequate to address uncertainties in the exposure and health effects databases (Table 7-6). Therefore, further refinements of the exposure estimate and POD are not warranted.

Table 7-6. Margins of exposure for 14 Azo Direct Dyes used in textiles

Exposure duration and route	Consumer products	Daily exposure (mg/kg-bw per day)	POD (mg/kg-bw per day)	MOEs
Daily oral	Textile objects (infants)	2.7×10^{-5}	26–300	≥ 963 000
Daily dermal	Textiles (personal apparel: adult)	0.0026	26–300	10000–115 000
Daily dermal	Textiles (baby	0.004	26–300	6500–75000

Exposure duration and route	Consumer products	Daily exposure (mg/kg-bw per day)	POD (mg/kg-bw per day)	MOEs
	sleeper: infant)			

Abbreviations: MOE, margin of exposure; POD, point of departure

Nine of the Azo Direct Dyes (Direct Black 56, Direct Red 31, Direct Red 81 triethanolamine salt, Direct Yellow 12, Direct Yellow 50, CAS RN 28706-21-0, CAS RN 71033-21-1, CAS RN 83221-56-1 and CAS RN 84878-17-1) were identified to be used as dyes in leather products. Exposure to these dyes in leather products is considered to be short term and intermittent. Therefore, the margins of exposure derived for these dyes used in textiles are considered to be protective for individuals using leather products.

Seven of the Azo Direct Dyes (Direct Blue 71, Direct Red 31, Direct Yellow 11, Direct Yellow 11 lithium salt, Direct Yellow 12, Direct Yellow 34 and Direct Yellow 50) were identified to be used as dyes in paper products. It is recognised that young children (predominately 0.5 – 4 years of age) may be infrequently exposed to these dyes from incidental ingestion of paper products. However, available data do not indicate acute toxicity to be of health concern for the Azo Direct Dyes, therefore, risk to young children from exposure to these dyes in paper products is considered to be low.

Two Azo Direct Dyes (Direct Blue 71 and Direct Yellow 11 lithium salt) were identified as having colourant uses in food packaging materials. As exposure to these dyes from direct food contact is not expected to be significant, risk to human health is considered to be low for this use.

Exposure to the remaining 43 Azo Direct Dyes has not been identified; accordingly, risk to human health from exposure to these dyes is not expected.

7.3.2 Azo Reactive Dyes

For the general population of Canada, environmental media are not considered to be a significant source of exposure to the Azo Reactive Dyes; therefore risk to human health from this source is considered to be low. Use of Reactive Black 5, Reactive Black 158 and Reactive Blue 225 as covalently bound dyes in textiles is not expected to be a significant source of exposure, therefore the risk to human health is considered to be low for these substances. For the remaining five Azo Reactive Dyes, available information did not identify sources of current exposure for the general population of Canada, therefore risk to human health is not expected for these substances.

7.3.3 Uncertainties in Characterization of Risk to Human Health

Azo Direct Dyes

Direct and prolonged dermal and oral exposure may occur to 14 Azo Direct Dyes via contact with textiles. There is uncertainty associated with the POD as it was used to characterize the risks for both high potency substances (such as aniline-based dyes) and low potency substances (such as sulfonated aromatic amine-based dyes). The relative absorption for the oral and dermal routes of exposure was also not distinguished. However, this risk characterization approach is considered reasonably conservative as the POD range covers the most potent substances among these dyes by including data for Acid Red 1. In comparison to the 14 dyes, Acid Red 1 is smaller, suggesting it may have higher bioavailability and hence higher toxic potential. Also, because the 14 dyes are water soluble, ionized substances, it is unlikely that they would have higher absorption via the dermal route relative to the oral route.

Some of these dyes contain unsulfonated aromatic amines whose health effects are unknown. 4-amino-1,3-Benzenediol contains two hydroxyl- groups, similar to a metabolite of aniline; this aromatic amine is unlikely to be more potent than aniline. 1,3-bis(4-amino-3-methylphenyl)urea, 1,3-bis(4-amino-2-methoxy-5-methylphenyl)urea and 4-amino-N-(4-aminophenyl)benzenesulfonamide have molecular weights two fold or more greater than that of aniline, whereas 1,4-Naphthalenediamine and 4-Amino-2-methoxy-5-methylaniline are relatively smaller molecules, only slightly larger than aniline. Although the toxicological potencies of these aromatic amines relative to aniline remain unknown, based on available data it is expected that their potential critical effect levels would be within the range of those for aniline, as the lowest LOEL identified in the OECD QSAR Toolbox (2013) for non-benzidine aromatic amines was 6 mg/kg-bw per day, whereas the LOAEL for aniline was 7.2 mg/kg-bw per day. In addition, aniline-based disazo dyes in this subgroup could potentially release aniline at a 1:2 ratio, and some polyazo dyes could release three unsulfonated aromatic amines. Although *in vivo* azo bond reductive cleavage efficiency for these disazo and polyazo dyes is unknown, even if it occurs rapidly and completely, it would result in potential critical effect levels within two to three folds of those for Acid Red 1. As the MOEs for the 14 dyes were sufficiently large to address these uncertainties, further refinement (such as refining dermal absorption and the point of departure for risk characterization) was not considered necessary.

It is recognized that Direct Red 81 triethanolamine salt may have additional health effects that could be solely attributed to triethanolamine. This could be relevant to individuals occupationally exposed to Direct Red 81 triethanolamine salt; however, the focus of this assessment is on the general population who may be exposed to finished dyed textile materials or leather products and are therefore not expected to be exposed to triethanolamine.

Azo Reactive Dyes

It is recognized that some of the Azo Reactive Dyes may have sensitization potential for individuals directly exposed to the concentrated dyes; however the focus of this assessment is on the general population who may be exposed to finished dyed materials and are therefore not expected to be exposed directly to concentrated azo reactive dyes.

7.3.4 Azo Direct Dyes with Effects of Concern

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

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 Azo Direct and Azo Reactive Dyes evaluated in this assessment. It is concluded that the 61 Azo Direct Dyes and the eight Azo Reactive Dyes do not meet the criteria under paragraph 64*(a)* or 64*(b)* of CEPA 1999, as they are not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

Based on the information presented in this Screening Assessment, it is concluded that the 61 Azo Direct Dyes and the 8 Azo Reactive Dyes 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 61 Azo Direct Dyes and 8 Azo Reactive Dyes do not meet any of the criteria set out in section 64 of CEPA 1999.

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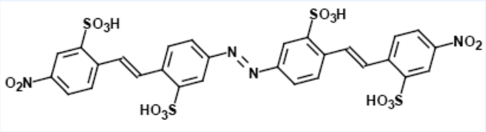
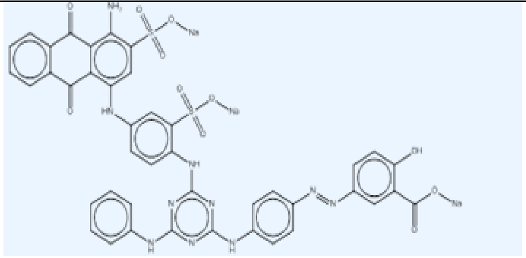
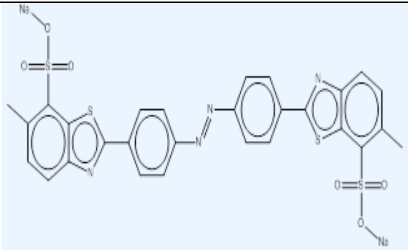
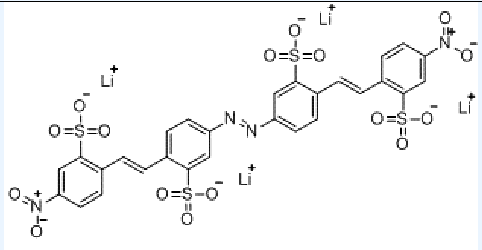
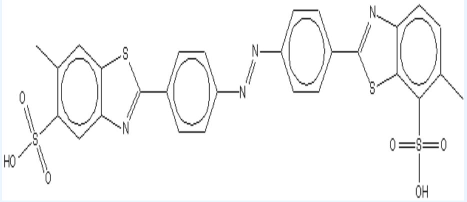
Appendices

Appendix A. Supplementary Data Tables

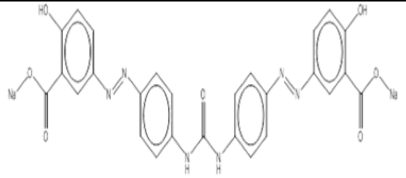
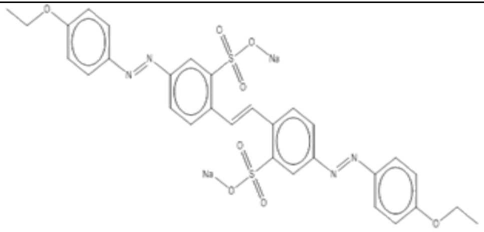
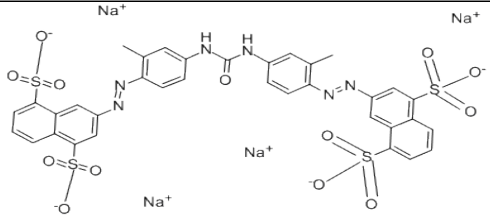
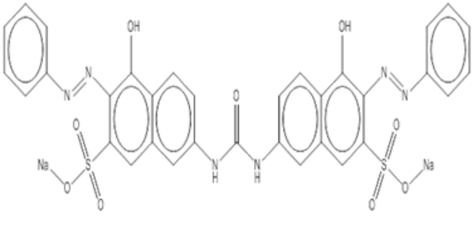
Table A-1. Structural identity information for the five Monoazo Direct Dyes

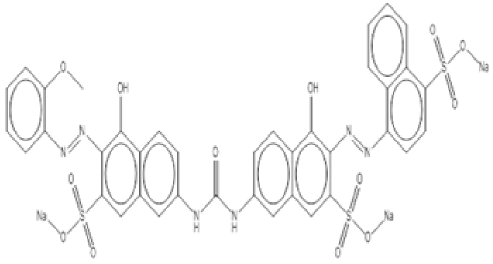
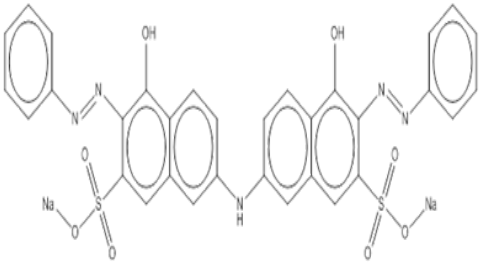
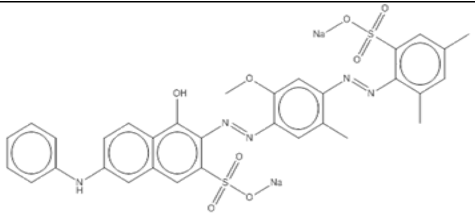
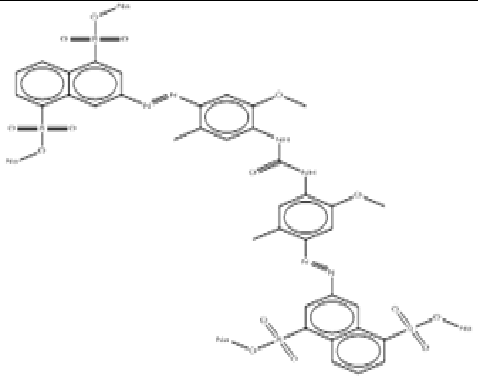
CAS RN	C.I. name or common name	Chemical structure and chemical formula	Molar weight (g/mol)
1325-37-7	Direct Yellow 11	 $C_{24}H_{10}N_4Na_2O_6S_4$ (estimate)	624
6471-09-6	Direct Green 28	 $C_{42}H_{27}N_{10}Na_3O_{11}S_2$	980
10114-47-3	Direct Yellow 28	 $C_{28}H_{18}N_4Na_2O_6S_4$	680
65150-80-3	Direct Yellow 11 lithium salt	 $C_{24}H_{10}N_4Li_2O_6S_4$ (estimate)	592

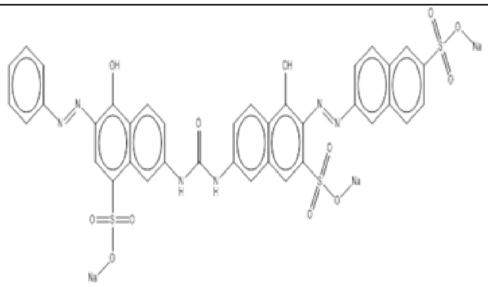
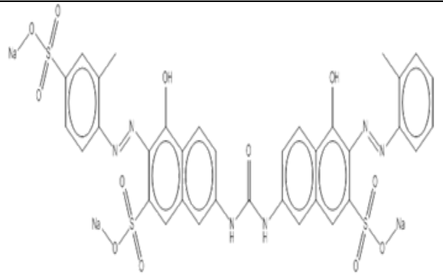
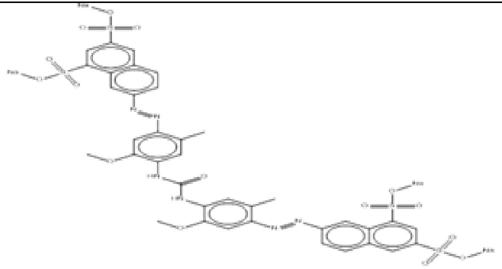
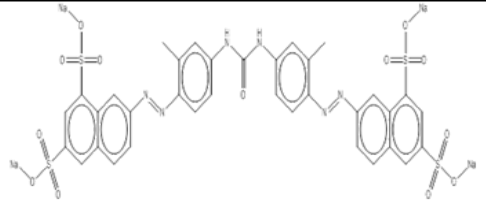
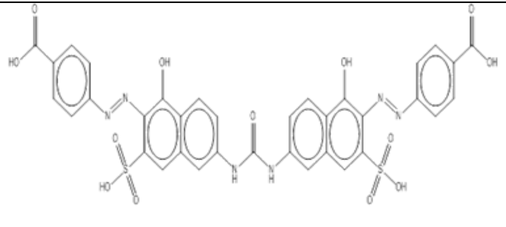
CAS RN	C.I. name or common name	Chemical structure and chemical formula	Molar weight (g/mol)
71033-21-1	NA	 $C_{28}H_{20}N_4Na_2O_6S_4$	682

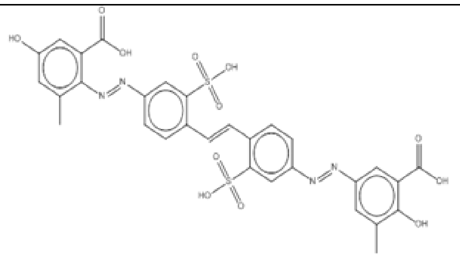
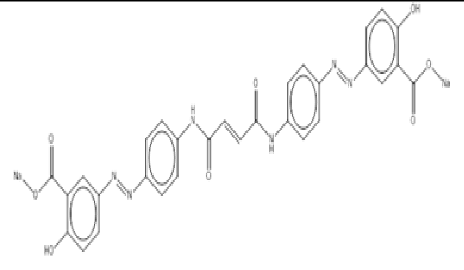
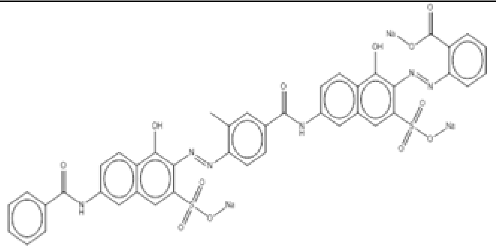
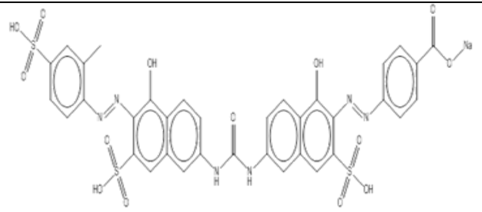
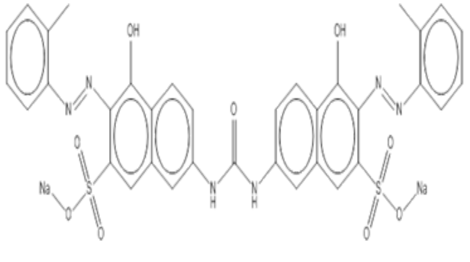
Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index; NA, not available

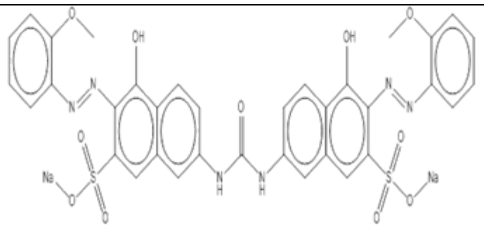
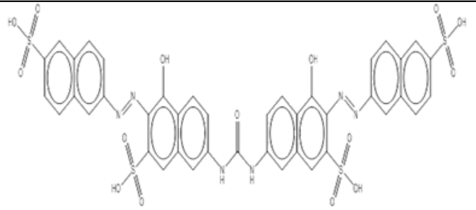
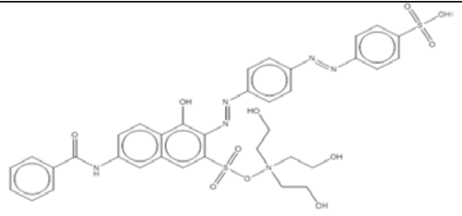
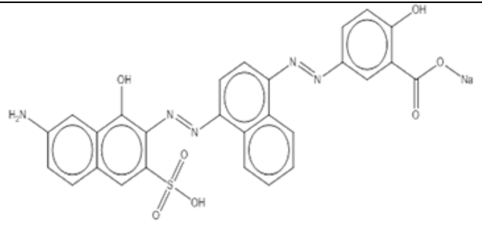
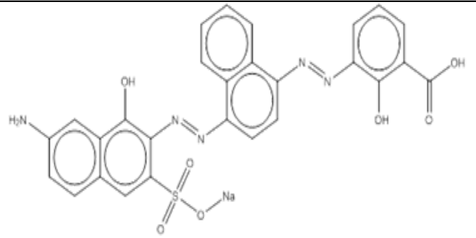
Table A-2. Structural identity information for the 37 Disazo Direct Dyes

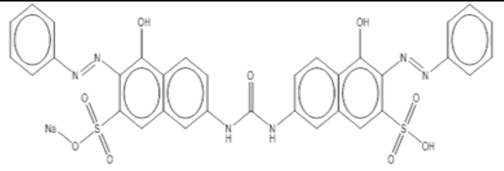
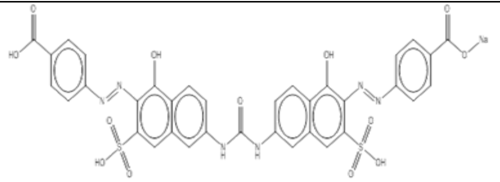
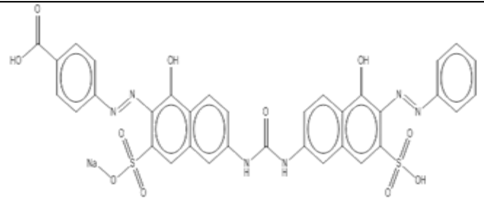
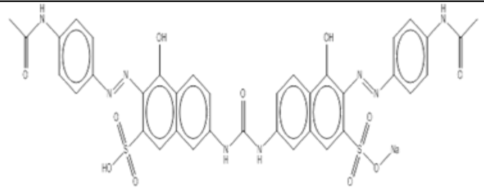
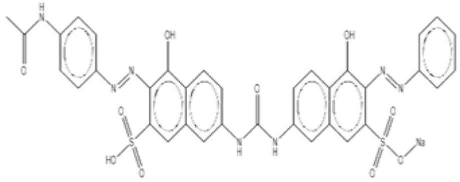
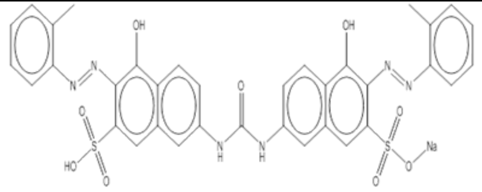
CAS RN	C.I. name or common name	Chemical structure and chemical formula	Molar weight (g/mol)
2829-42-7	Direct Yellow 26	 $C_{27}H_{18}N_6Na_2O_7$	584
2870-32-8	Direct Yellow 12	 $C_{30}H_{26}N_4Na_2O_8S_2$	680
3214-47-9	Direct Yellow 50	 $C_{35}H_{24}N_6Na_4O_{13}S_4$	956
3626-36-6	Direct Orange 26	 $C_{33}H_{22}N_6Na_2O_9S_2$	756

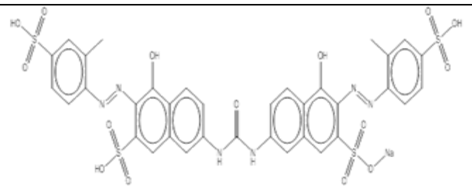
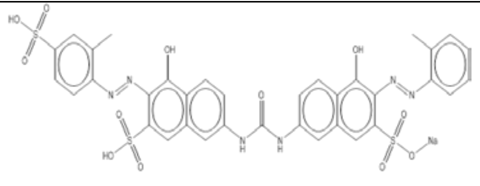
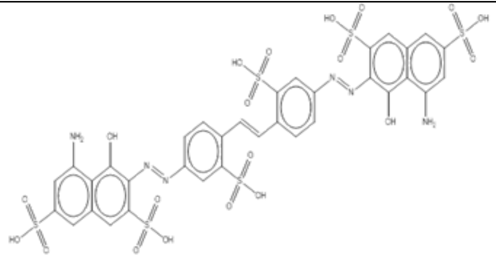
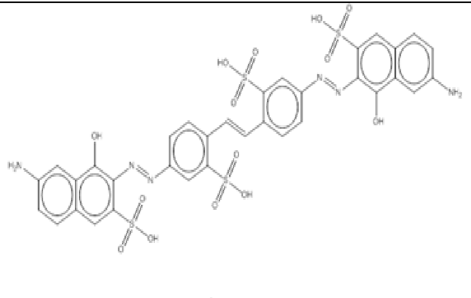
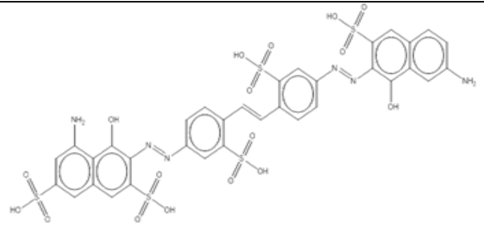
CAS RN	C.I. name or common name	Chemical structure and chemical formula	Molar weight (g/mol)
3687-80-7	Direct Red 26	 $C_{38}H_{25}N_6Na_3O_{13}S_3$	938
5001-72-9	Direct Red 31	 $C_{32}H_{21}N_5Na_2O_8S_2$	713
5489-77-0	Direct Violet 51	 $C_{32}H_{27}N_5Na_2O_8S_2$	719
6420-33-3	Direct Yellow 34	 $C_{37}H_{28}N_6Na_4O_{15}S_4$	1016

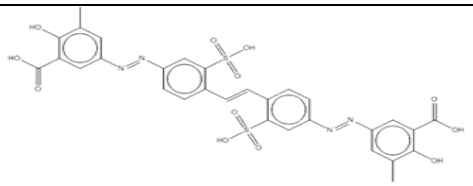
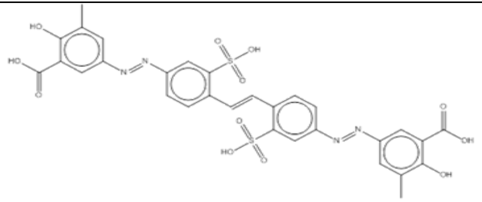
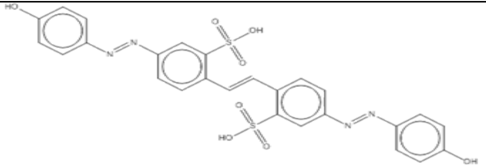
CAS RN	C.I. name or common name	Chemical structure and chemical formula	Molar weight (g/mol)
6420-41-3	Direct Red 4	 $C_{37}H_{23}N_6Na_3O_{12}S_3$	908
6420-43-5	Direct Red 62	 $C_{35}H_{25}N_6Na_3O_{12}S_3$	886
12217-64-0	Direct Orange 72	 $C_{37}H_{28}N_6Na_4O_{15}S_4$	1016
28706-21-0	NA	 $C_{35}H_{24}N_6Na_4O_{13}S_4$	956
38801-08-0	NA	 $C_{35}H_{24}N_6O_{13}S_2$	1397

CAS RN	C.I. name or common name	Chemical structure and chemical formula	Molar weight (g/mol)
53523-90-3	NA	 $C_{30}H_{20}Li_4N_4O_{12}S_2$	720
72139-21-0	NA	 $C_{30}H_{20}N_6Na_2O_8$	638
72152-50-2	NA	 $C_{42}H_{27}N_6Na_3O_{12}S_2$	940
72245-49-9	NA	 $C_{35}H_{26}N_6Na_2O_{14}S_3$	938
72749-87-2	NA	 $C_{35}H_{28}N_6Na_2O_9S_2$	784

CAS RN	C.I. name or common name	Chemical structure and chemical formula	Molar weight (g/mol)
72749-88-3	NA	 $C_{35}H_{26}N_6Na_2O_{11}S_2$	816
72869-93-3	NA	 $C_{35}H_{28}N_{10}O_{23}S_4$	724
75768-93-3	Direct Red 81 triethanolamine salt	 $C_{29}H_{21}N_5O_8S_2\cdot 2C_6H_{15}NO$	813
83221-53-8	NA	 $C_{27}H_{17}N_5Na_2O_7S_2$	601
83221-54-9	NA	 $C_{27}H_{17}N_5Na_2O_7S$	601

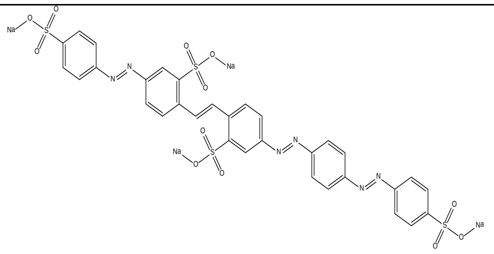
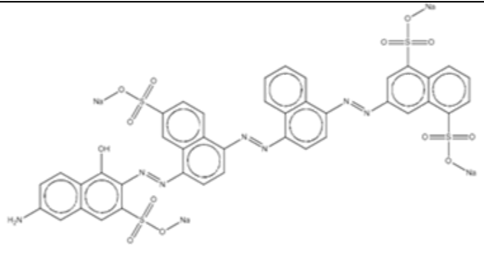
CAS RN	C.I. name or common name	Chemical structure and chemical formula	Molar weight (g/mol)
83221-56-1	NA	 $C_{33}H_{24}N_6NaO_9S_2$	736
83221-73-2	NA	 $C_{33}H_{24}N_6NaO_{13}S_2$	888
83221-74-3	NA	 $C_{33}H_{24}N_6NaO_{11}S_2$	822
83232-28-4	NA	 $C_{37}H_{30}N_8NaO_{11}S_2$	850
83232-29-5	NA	 $C_{35}H_{25}N_7Na_2O_{10}S_2$	813
83232-30-8	NA	 $C_{35}H_{28}N_6Na_2O_9S_2$	786

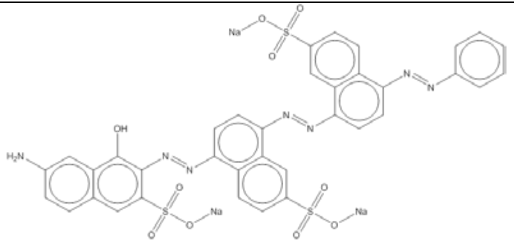
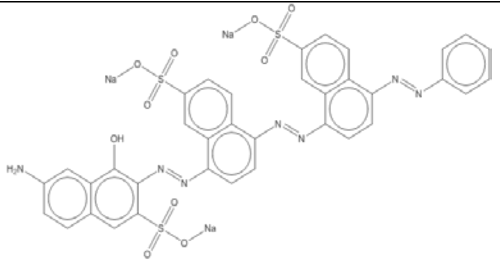
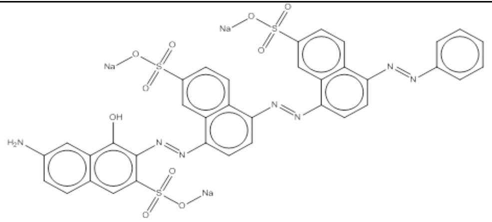
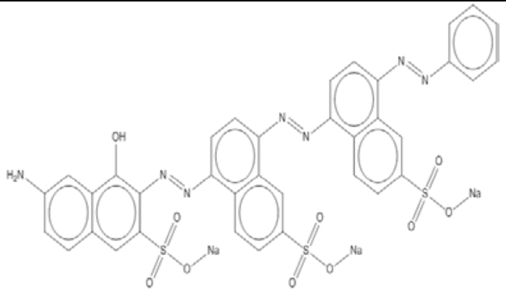
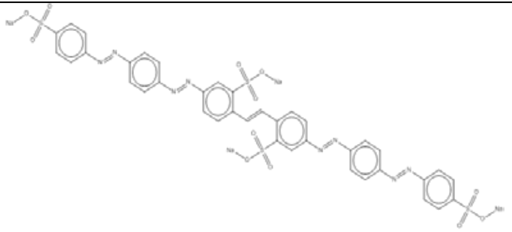
CAS RN	C.I. name or common name	Chemical structure and chemical formula	Molar weight (g/mol)
83232-31-9	NA	 $C_{35}H_{28}N_6Na_2O_{15}S_4$	924
83232-32-0	NA	 $C_{35}H_{28}N_6NaO_{15}S_3$	886
83783-94-2	NA	 $C_{39}H_{33}Li_2N_7Na_4O_{22}S_6$	1249
83783-95-3	NA	 $C_{39}H_{35}LiN_7Na_3O_{16}S_4$	1061
83783-96-4	NA	 $C_{39}H_{34}Li_2N_7Na_3O_{19}S_5$	1147

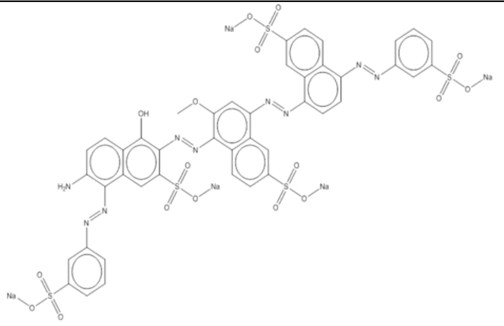
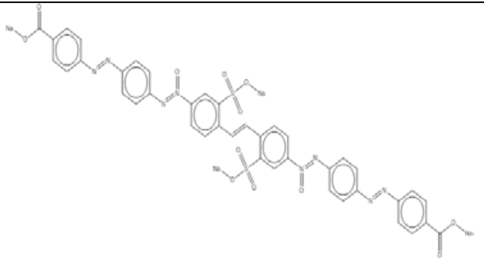
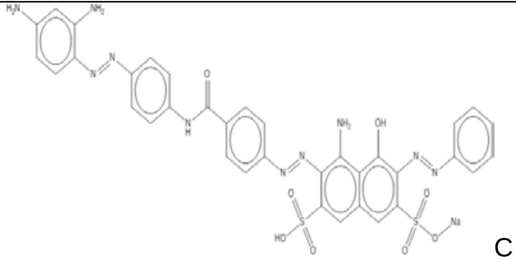
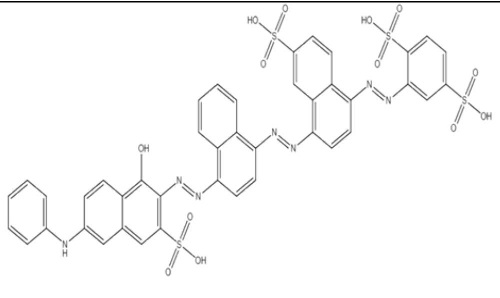
CAS RN	C.I. name or common name	Chemical structure and chemical formula	Molar weight (g/mol)
83783-99-7	NA	 $C_{35}H_{35}LiN_5NaO_{14}S_2$	843
85269-31-4	NA	 $C_{30}H_{24}N_4O_{12}S_2 \cdot xC_6H_{15}NO_3$	812
110152-63-1	NA	 $C_{26}H_{20}N_4O_8S_2 \cdot xLi \cdot xNa$	802

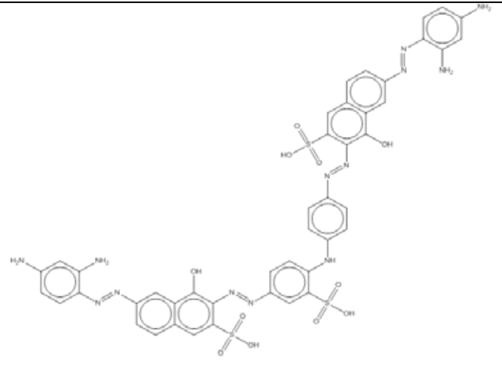
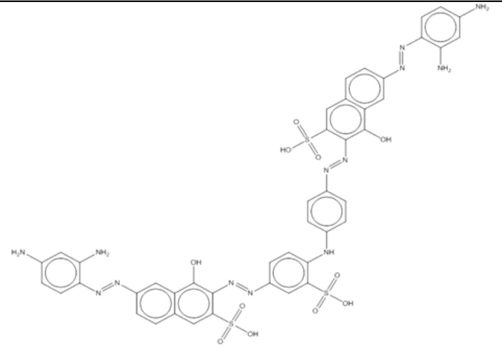
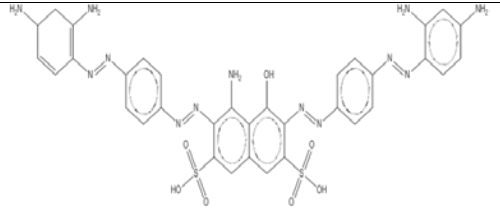
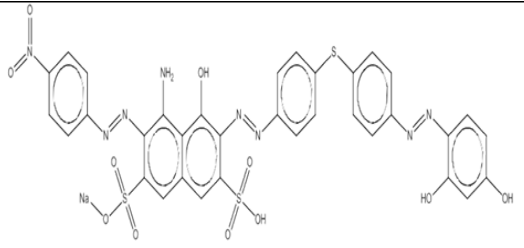
Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index; NA, not available

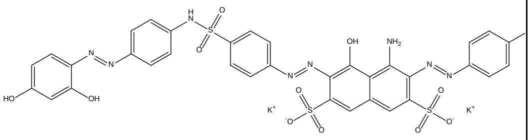
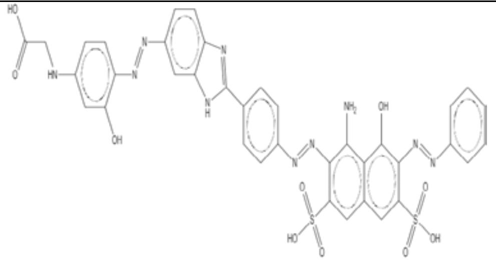
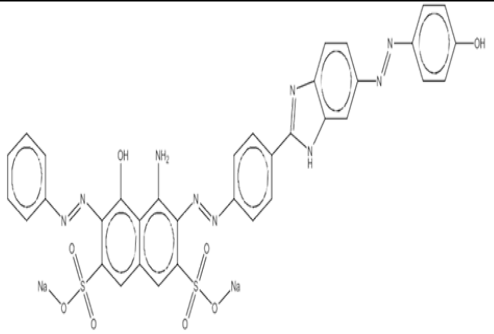
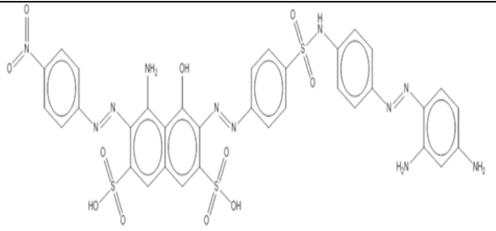
Table A-3. Structural identity information for the 19 individual Polyazo Direct Dyes

CAS RN	C.I. name or common name	Chemical structure and chemical formula	Molar weight (g/mol)
1325-54-8	Direct Orange 39	 $C_{32}H_{24}N_6O_{10}S_2 \cdot Li \cdot Na$	990
4399-55-7	Direct Blue 71	 $C_{40}H_{23}N_7Na_4O_{13}S_4$	1029

CAS RN	C.I. name or common name	Chemical structure and chemical formula	Molar weight (g/mol)
6406-87-7	NA	 $C_{36}H_{22}N_7Na_3O_{10}S_3$	877
6476-10-4	NA	 $C_{36}H_{22}N_7Na_3O_{10}S_3$	877
10134-33-5	Direct Black 56	 $C_{36}H_{22}N_7Na_3O_{10}S_3$	877
10482-42-5	NA	 $C_{36}H_{22}N_7Na_3O_{10}S_3$	877
32829-81-5	NA	 $C_{38}H_{24}N_8Na_4O_{12}S_4$	1004

CAS RN	C.I. name or common name	Chemical structure and chemical formula	Molar weight (g/mol)
71767-19-6	NA	 $C_{43}H_{26}N_9Na_5O_{17}S_5$	1050
71873-49-9	NA	 $C_{40}H_{24}N_8Na_4O_{12}S_2$	964
72245-56-8	NA	 $C_{35}H_{26}N_{10}Na_2O_8S_2$	824
75150-14-0	NA	 $C_{42}H_{33}N_9Na_2O_{13}S_4$	1046

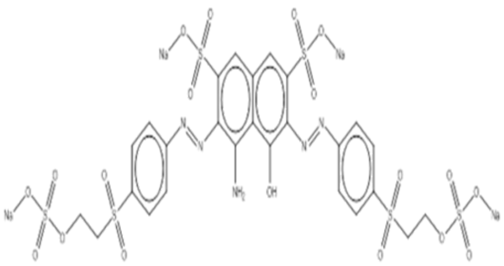
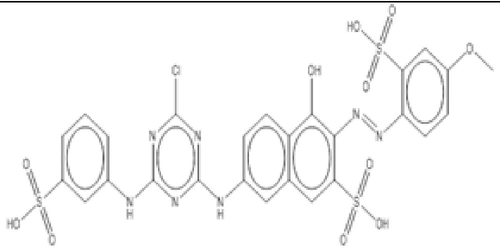
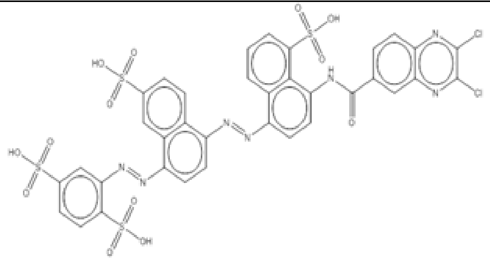
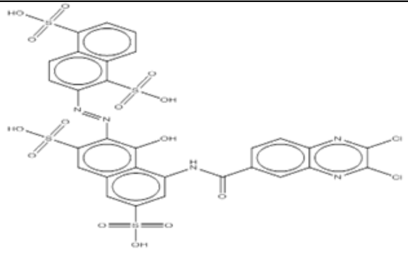
CAS RN	C.I. name or common name	Chemical structure and chemical formula	Molar weight (g/mol)
83221-68-5	NA	 $C_{44}H_{32}Li_3N_{13}O_{11}S_3$	635
83221-69-6	NA	 $C_{44}H_{35}Li_2N_{13}NaO_{11}S_3$	1050
83221-72-1	NA	 $C_{34}H_{27}LiN_{13}NaO_7S_2$	823
84878-16-0	NA	 $C_{34}H_{22}N_8Na_2O_{11}S_3$	860

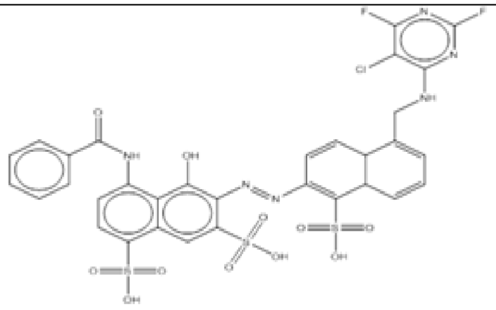
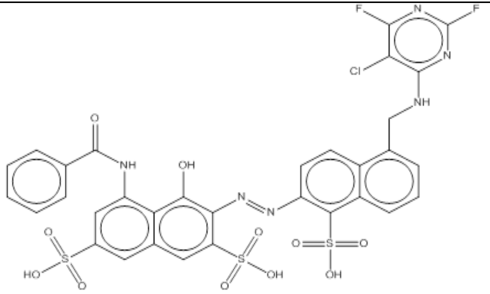
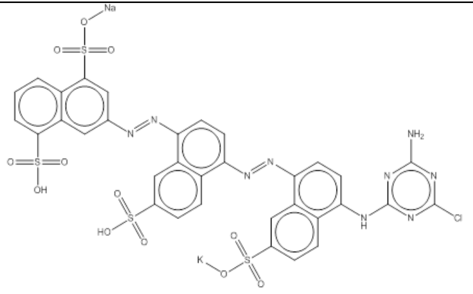
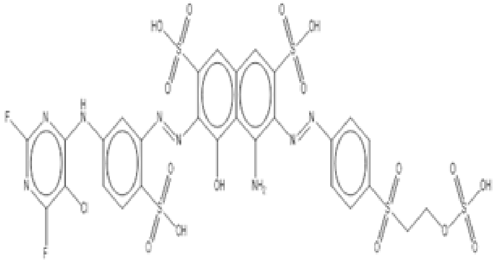
CAS RN	C.I. name or common name	Chemical structure and chemical formula	Molar weight (g/mol)
84878-17-1	NA	 $C_{34}H_{23}K_2N_9O_{13}S_3$	939
85169-18-2	NA	 $C_{37}H_{28}N_{10}O_{10}S_2 \cdot 3C_4H_{11}NO$	801
93803-37-3	NA	 $C_{35}H_{23}N_9Na_2O_8S_2$	807
102082-94-0	NA	 $C_{34}H_{27}N_{11}O_{11}S_3Li$	873

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index; NA, not available

Table A-4. Structural identity information for the eight Azo Reactive Dyes

CAS RN	C.I. name or common name	Chemical structure and chemical formula	Molar weight (g/mol)

CAS RN	C.I. name or common name	Chemical structure and chemical formula	Molar weight (g/mol)
17095-24-8	Reactive Black 5	 $C_{26}H_{21}N_5Na_4O_{19}S_6$	991
59641-46-2	NA	 $C_{26}H_{20}ClN_7O_{11}S_3$	738
83399-85-3	NA	 $C_{35}H_{17}Cl_2Li_2N_7Na_2O_{13}S$	1002
83400-10-6	NA	 $C_{29}H_{13}C_{l2}Li_2N_5Na_2O_{14}S_4$	914

CAS RN	C.I. name or common name	Chemical structure and chemical formula	Molar weight (g/mol)
83400-11-7	Reactive Black 158	 $C_{32}H_{18}ClF_2LiN_6Na_2O_{11}S_3$	885
83400-12-8	NA	 $C_{32}H_{18}ClF_2LiN_6Na_2O_{11}S_3$	885
85586-78-3	NA	 $C_{33}H_{18}ClK_2N_9Na_2O_{12}S_4$	964
108624-00-6	Reactive Blue 225	 $C_{28}H_{21}ClF_2LiN_8O_{16}S_5$	1015

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index; NA, not available

Table A-5. Available experimental data for physical and chemical properties of individual Azo Direct Dyes

C.I. name or common name (CAS RN)	Property	Value	Reference
Direct Yellow 11 (1325-37-7)	Water solubility	200 mg/L (RT)	Wang et al. 2008
Direct Yellow 11 (1325-37-7)	Melting/decomposition point	Decomposes without melting when heated to 390°C	GuideChem 2013
Direct Yellow 11 lithium salt (65150-80-3)	Melting/decomposition point	< −12°C	BASF 2004
Direct Yellow 11 lithium salt (65150-80-3)	Water solubility	Miscible	BASF 2004
Direct Yellow 12 (2870-32-8)	Water solubility	25 mg/L	Ghaedi et al. 2012
Direct Yellow 12 (2870-32-8)	Water solubility	25–125 mg/L (27°C)	Khaled et al. 2009
Direct Yellow 12 (2870-32-8)	Water solubility	25–40 mg/L (27°C)	Ghaedi et al. 2013
Direct Orange 26 (3626-36-6)	Water solubility	30 mg/mL	Shen et al. 2001
Direct Violet 51 (5489-77-0)	Water solubility	20 mg/mL	Green 1990
Direct Orange 39 (1325-54-8)	Water solubility	20 mg/mL	Vujevic et al. 2004
Direct Blue 71 (4399-55-7)	Water solubility	100 mg/mL	Oranusi and Ogugbue 2002
Direct Blue 71 (4399-55-7)	Water solubility	40 mg/mL	Green 1990
Direct Blue 71 (4399-55-7)	Water solubility	60 mg/mL	Franciscon et al. 2012
Direct Blue 71 (4399-55-7)	Melting/decomposition point	175°C	Acros Organics 1997

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

Table A-6. Available experimental data for physical and chemical properties of individual Azo Reactive Dyes

C.I. name or common name (CAS RN)	Property	Value	Reference
Reactive Black 5 (17095-24-8)	Water solubility	> 2000 mg/L	ETAD 2008
Reactive Black 5 (17095-24-8)	Water solubility	100 mg/L	Chompuchan et al. 2010
Reactive Black 5 (17095-24-8)	Water solubility	40 mg/L	Dojcinovic et al. 2012
Reactive Black 5 (17095-24-8)	Water solubility	200 mg/L	Kumar et al. 2012
Reactive Black 5 (17095-24-8)	Water solubility	200 mg/L	Green 1990
Reactive Black 5 (17095-24-8)	Water solubility	60 mg/mL	Franciscon et al. 2012
Reactive Black 5 (17095-24-8)	Melting/decomposition point	> 300°C	Rohilla et al. 2012
Reactive Blue 225 (108624-00-6)	Water solubility	Very soluble (> 15%)	Technology Supplies Ltd. 2012

Table A-7. Summary of modelled data relevant to the persistence of Monoazo Direct Dyes[a]

Environmental compartment	Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (d)
Air	Atmospheric oxidation	AOPWIN 2010[b]	$t_{1/2}$ = 0.053–1.184 d	< 2
Air	Ozone reaction	AOPWIN 2010[b]	N/A[c]	N/A
Water	Hydrolysis	HYDROWIN 2010[b]	Not in training set	N/A
Water	Primary biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 4: Expert survey (qualitative results)	1.926–2.782[d] (biodegrades slowly)	≥ 182
Water	Ultimate biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 3: Expert	0.362–1.626[d] (biodegrades slowly)	≥ 182

Environmental compartment	Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (d)
		survey (qualitative results)		
Water	Biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 5: MITI linear probability	−ITI linear prob[e] (biodegrades slowly)	≥ 182
Water	Biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 6: MITI non-linear probability	0[e] (biodegrades very slowly)	≥ 182
Water	Biodegradation (aerobic)	DS TOPKAT ©2005–2009 Probability	N/A[c]	N/A[c]
Water	Biodegradation (aerobic)	CATALOGIC 2012 % BOD	% BOD = 0–20 (biodegrades slowly)	≥ 182

Abbreviations: BOD, biological oxygen demand; MITI, Ministry of International Trade and Industry (Japan); N/A, not applicable; $t_{1/2}$, half-life

[a] Substances used in this summary include the following CAS RNs: 1325-37-7, 6471-09-6, 10114-47-3, 65150-80-3, 71033-21-1.

[b] EPI Suite (2012).

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

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

[e] Output is a probability score.

Table A-8. Summary of modelled data relevant to the persistence of Disazo Direct Dyes[a]

Environmental compartment	Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (d)
Air	Atmospheric oxidation	AOPWIN 2010[b]	$t_{1/2}$ = 0.056–0.632 d	< 2
Air	Ozone reaction	AOPWIN 2010[b]	N/A[c]	N/A
Water	Hydrolysis	HYDROWIN 2010[b]	Not in training set	N/A
Water	Primary biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 4: Expert survey (qualitative results)	2.505–3.390[d] (biodegrades slowly)	≥ 182
Water	Ultimate biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 3: Expert survey	0.690–1.650[d] (biodegrades slowly)	≥ 182

Environmental compartment	Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (d)
		(qualitative results)		
Water	Ultimate biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 5: MITI linear probability	−0.043 to −1.644[e] (biodegrades slowly)	≥ 182
Water	Ultimate biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 6: MITI non-linear probability	0[e] (biodegrades very slowly)	≥ 182
Water	Ultimate biodegradation (aerobic)	DS TOPKAT ©2005–2009 Probability	N/A[c]	N/A[c]
Water	Ultimate biodegradation (aerobic)	CATALOGIC 2012 % BOD	% BOD = 0–20 (biodegrades slowly)	≥ 182

Abbreviations: BOD, biological oxygen demand; MITI, Ministry of International Trade and Industry (Japan); N/A, not applicable; $t_{1/2}$, half-life

[a] Substances used in this summary include the following CAS RNs: 2829-42-7, 2870-32-8, 3214-47-9, 3626-36-6, 3687-80-7, 5001-72-9, 5489-77-0, 6420-33-3, 6420-41-3, 6420-43-5, 12217-64-0, 28706-21-0, 38801-08-0, 53523-90-3, 72139-21-0, 72152-50-2, 72245-49-9, 72749-87-2, 72749-88-3, 72869-93-3, 75768-93-3, 83221-53-8, 83221-54-9, 83221-56-1, 83221-73-2, 83221-74-3, 83232-28-4, 83232-29-5, 83232-30-8, 83232-31-9, 83232-32-0, 83783-94-2, 83783-95-3, 83783-96-4, 83783-99-7, 85269-31-4, 110152-63-1.

[b] EPI Suite (2012).

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

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

[e] Output is a probability score.

Table A-9. Summary of modelled data relevant to the persistence of Polyazo Direct Dyes[a]

Environmental compartment	Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (d)
Air	Atmospheric oxidation	AOPWIN 2010[b]	$t_{1/2}$ = 0.030–1.007 d	< 2
Air	Ozone reaction	AOPWIN 2010[b]	N/A[c]	N/A
Water	Hydrolysis	HYDROWIN 2010[b]	Not in training set	N/A
Water	Primary biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 4: Expert survey (qualitative results)	1.648–2.953[d] (biodegrades slowly)	≥ 182
Water	Ultimate biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 3: Expert survey	−0.449 to 1.120[d] (biodegrades slowly)	≥ 182

Environmental compartment	Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (d)
		(qualitative results)		
Water	Ultimate biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 5: MITI linear probability	−3.171 to −0.712[e] (biodegrades slowly)	≥ 182
Water	Ultimate biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 6: MITI non-linear probability	0[e] (biodegrades very slowly)	≥ 182
Water	Ultimate biodegradation (aerobic)	DS TOPKAT ©2005–2009 Probability	N/A[c]	N/A[c]
Water	Ultimate biodegradation (aerobic)	CATALOGIC 2012 % BOD	% BOD = 0–20 (biodegrades slowly)	≥ 182

Abbreviations: BOD, biological oxygen demand; MITI, Ministry of International Trade and Industry (Japan); N/A, not applicable; $t_{1/2}$, half-life

[a] Substances used in this summary include the following CAS RNs: 1325-54-8, 4399-55-7, 6406-87-7, 6476-10-4, 10134-33-5, 10482-42-5, 32829-81-5, 71767-19-6, 71873-49-9, 72245-56-8, 75150-14-0, 83221-68-5, 83221-69-6, 83221-72-1, 84878-16-0, 84878-17-1, 85169-18-2, 93803-37-3, 102082-94-0.

[b] EPI Suite (2012).

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

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

[e] Output is a probability score.

Table A-10. Summary of modelled data relevant to the persistence of Azo Reactive Dyes

Environmental compartment	Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (d)
Air	Atmospheric oxidation	AOPWIN 2010[b]	$t_{1/2}$ = 0.029–4.540 d	≥ 2
Air	Ozone reaction	AOPWIN 2010[b]	N/A[c]	N/A
Water	Hydrolysis	HYDROWIN 2010[b]	Not in training set	N/A
Water	Primary biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 4: Expert survey (qualitative results)	2.067–2.770[d] (biodegrades slowly)	≥ 182
Water	Ultimate biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 3: Expert survey (qualitative results)	−0.390 to 1.097[d] (biodegrades slowly)	≥ 182

Environmental compartment	Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (d)
Water	Ultimate biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 5: MITI linear probability	−2.288 to −1.305[e] (biodegrades slowly)	≥ 182
Water	Ultimate biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 6: MITI non-linear probability	0[e] (biodegrades very slowly)	≥ 182
Water	Ultimate biodegradation (aerobic)	DS TOPKAT ©2005–2009 Probability	N/A[c]	N/A[c]
Water	Ultimate biodegradation (aerobic)	CATALOGIC 2012 % BOD	% BOD = 0–20 (biodegrades slowly)	≥ 182

Abbreviations: BOD, biological oxygen demand; MITI, Ministry of International Trade and Industry (Japan); N/A, not applicable; $t_{1/2}$, half-life

[a] Substances used in this summary include the following CAS RNs: 17095-24-8, 59641-46-2, 83399-85-3, 83400-10-6, 83400-11-7, 83400-12-8, 85586-78-3, 108624-00-6.

[b] EPI Suite (2012).

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

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

[e] Output is a probability score.

Table A-11. Available experimental data for aquatic toxicity of Azo Direct Dyes

C.I. name (CAS RN)	Test organism[a]	Type of test (duration)	Endpoint	Value (mg/L)	Reference
Direct Yellow 11 (1325-37-7)	*Pimephales promelas*	Acute (96 h)	NOEC	> 180	Little et al. 1974
Direct Yellow 11 lithium salt (65150-80-3)	*Daphnia magna*	Acute (96 h)	LC_{50}	100	BASF 2004
Direct Yellow 12 (2870-32-8)	*Pimephales promelas*	Acute (24 h)	LC_{50}	180	Little and Lamb 1973
Direct Yellow 12 (2870-32-8)	*Pimephales promelas*	Acute (48 h)	LC_{50}	130	Little and Lamb 1973
Direct	*Pimephales*	Chronic (4 d)	LC_{50}	125	Little and Lamb

C.I. name (CAS RN)	Test organism[a]	Type of test (duration)	Endpoint	Value (mg/L)	Reference
Yellow 12 (2870-32-8)	*promelas*				1973
Direct Yellow 50 (3214-47-9)	*Oryzias latipes*	Acute (96 h)	LC_{50}	600	CHRIP ©2008
Direct Yellow 50 (3214-47-9)	*Leuciscus idus*	Acute (48 h)	LC_{100}	140–200	Hamburger et al. 1977
Direct Yellow 50 (3214-47-9)	*Leuciscus idus*	Acute (48 h)	LC_{100}	> 500	Hamburger et al. 1977
Direct Yellow 50 (3214-47-9)	*Oncorhynchus mykiss*	Acute (48 h)	LC_{100}	> 500	Hamburger et al. 1977
Direct Yellow 50 (3214-47-9)	*Oncorhynchus mykiss*	Acute (48 h)	LC_{100}	> 1000	Hamburger et al. 1977
Direct Yellow 50 (3214-47-9)	*Leuciscus idus*	Acute (48 h)	LC_{50}	140–200	Hamburger et al. 1977
Direct Yellow 50 (3214-47-9)	*Phoxinus phoxinus*	Acute (48 h)	LC_{50}	> 1000	Hamburger et al. 1977
Direct Yellow 50 (3214-47-9)	*Phoxinus phoxinus*	Acute (48 h)	LC_{50}	> 1700	Hamburger et al. 1977
Direct Yellow 50 (3214-47-9)	*Pimephales promelas*	Acute (24 h)	LC_{50}	> 180	Little et al. 1974
Direct Yellow 50 (3214-47-9)	*Pimephales promelas*	Acute (48 h)	LC_{50}	> 180	Little et al. 1974
Direct Yellow 50 (3214-47-9)	*Pimephales promelas*	Chronic (4 d)	LC_{50}	> 180	Little and Lamb 1973

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index; LC_{50}, the concentration of a substance that is estimated to be lethal to 50% of the test organisms; LC_{100}, the concentration of a substance that is estimated to be lethal to 100% 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)

[a] Common names for the species listed are as follows: fathead minnow (*Pimephales promelas*); freshwater water flea (*Daphnia magna*): Japanese killifish (*Oryzias latipes*); ide (*Leuciscus idus*); rainbow trout (*Oncorhynchus mykiss*); and common minnow (*Phoxinus phoxinus*).

Table A-12. Empirical data for aquatic toxicity for Azo Reactive Dyes

C.I. name (CAS RN)	**Test organism[a]**	**Type of test (duration)**	**Endpoint**	**Value (mg/L)[b]**	**Reference**
Reactive Black 5 (CAS 17095-24-8)	*Oryzias latipes*	Acute (48 h)	LC_{50}	1000	MITI 1992
Reactive Black 5 (CAS 17095-24-8)	*Oryzias latipes*	Acute (48 h)	LC_{50}	100–500	Øllgaard et al. 1998
Reactive Black 5 (CAS 17095-24-8)	*Danio rerio*	Chronic (4 d)	LC_{50}	> 500	ETAD 2008
Reactive Black 5 (CAS 17095-24-8)	*Daphnia magna*	Acute (48 h)	EC_{50}	> 128	ETAD 2008
Reactive Black 5 (CAS 17095-24-8)	*Daphnia magna*	Acute (48 h)	EC_{50}	> 1000	CHRIP ©2008
Reactive Black 5 (CAS 17095-24-8)	*Daphnia magna*	Chronic (21 d)	EC_{50}	> 20	CHRIP ©2008
Reactive Black 5 (CAS 17095-24-8)	*Daphnia magna*	Chronic (21 d)	NOEC	1.3	CHRIP ©2008
Reactive Black 5 (CAS 17095-24-8)	*Oryzias latipes*	Acute (96 h)	LC_{50}	> 100	CHRIP ©2008
Reactive Black 5 (CAS 17095-24-8)	*Oryzias latipes*	Chronic (14 d)	LC_{50}	> 100	CHRIP ©2008
Reactive Black 5 (CAS 17095-24-8)	*Oryzias latipes*	Chronic (14 d)	NOEC	> 100	CHRIP ©2008

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

[a] Common names for the species listed are as follows: fathead minnow (*Pimephales promelas*); freshwater water flea (*Daphnia magna*); Japanese killifish (*Oryzias latipes*); and zebrafish (*Danio rerio*).

Appendix B. Exposure Assessment

Appendix B1. Dermal and Oral Exposure via Contact with Textile Materials

Table B1-1: Estimated exposures to 14 Azo Direct Dyes via contact with textile materials

Product scenario	Daily exposure (mg/kg-bw per day)
Textiles; personal apparel (adult; dermal)	0.0026
Textiles; baby sleeper (infant; dermal)	0.004
Textiles (infant; oral)	2.7×10^{-5}

Summary B1: Exposure factors and algorithms for estimating exposure from textile materials

Dermal exposure from textile materials

Exposure estimate $\frac{SA\times AW\times SCF\times C\times M\times F}{BW} = \frac{SA\times AW\times SCF\times C\times M\times UF\times F\times P}{BW}$

Dermal exposure was estimated assuming 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 materials

Exposure estimate = $= \frac{SA\times AW\times C\times M\times F\times P}{BW} \frac{SA\times AW\times C\times M\times F}{BW}$

Oral exposure was estimated for an infant mouthing a textile object (e.g., blanket, textile toy) on a daily basis.

***SA*: Total surface area**

For dermal exposure (Health Canada 1998) = 18 200 cm^2 (adult; personal apparel); = 3020 cm^2 (infant; baby sleeper)

For oral exposure = 20 cm^2 (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.

***UF*: Uptake Fraction** = 1

In the absence of dermal absorption data, the dermal uptake fraction of these substances was conservatively assumed to be 1. Although the dermal uptake of these substances is likely less than 1, potentially there could be substantial absorption if skin bacteria cleaved azo bonds, thereby releasing aromatic amines, which could then be more readily absorbed

***F*: Frequency** = 1×/day

***BW*: Body weight** = 7.5 kg for infant, 70.9 kg for adult (Health Canada 1998)

***P*: Probability that a given Azo Direct Dye is present in textile** = 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. Presumably, there would be a higher prevalence in the use of non-EU22 amines and their dyes, compared to EU22 amines and related dyes, since the former are not prohibited. None of the Azo Direct Dyes used to dye textiles in Canada (i.e., Direct Green 28, Direct Orange 26, Direct Orange 39, Direct Red 81 triethanolamine salt, Direct Red 31, Direct Violet 51, Direct Yellow 12, Direct Yellow 28, Direct Yellow 50, Direct Black 56, CAS RN 28706-21-0, CAS RN 71033-21-1, CAS RN 83221-56-1 and CAS RN 84878-17-1) derive from EU22 amines; the prevalence of these dye is not clear because there is relatively limited product testing and monitoring on non-EU22 amines and associated dyes. Based on data available (Danish EPA 1998; Kawakami 2012; Health Canada 2013), the prevalence of certain non-EU22 amines was found to range from 0% to 23.7% (aniline). Since several dyes can derive from a given aromatic amine, the prevalence of an associated dye would be lower. Given the conservatism used in other parameters in this exposure scenario (e.g. full body coverage), the probability that a given Azo Direct Dye is present in a textile is assumed to be 10% in this Screening Assessment based on professional judgement. This is considered reasonable since the chances of an individual’s outfit containing a given Azo Direct Dye every day are low.

Appendix B2: Dermal Exposure via Contact with Leather Products

Table B2-1: Estimated exposures to nine Azo Direct Dyes from dermal contact with leather products

Product scenario	Per event exposure (mg/kg-bw)
Shoes	$5.8 \times 10^{-\cdot}$
Boots	$1.9 \times 10^{-\cdot}$
Gloves	$2.1 \times 10^{-\cdot}$
Jackets and coats	$7.7 \times 10^{-\cdot}$
Trousers	$5.0 \times 10^{-\cdot}$
Furniture	$2.3 \times 10^{-\cdot}$
Toys	$4.0 \times 10^{-\cdot}$

Summary B2: Exposure factors and algorithms for estimating exposure from leather products

Dermal exposure from leather products

Exposure estimate = $\frac{SA \times AW \times SCF \times C \times M}{BW}$

Prolonged 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 prolonged contact with the infant's palms can occur when playing with the toy. As a conservative approach, exposure is assumed for all products. The exposure estimates presented below are based on conservative assumptions, as well as not taking into account a final application of a polyurethane sealant coating, which would further reduce the consumer's dermal exposure to the leather dye.

***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** = 0.39 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 1 year and is available for dermal exposure.

***BW*: Body weight** = 7.5 kg for infant, 70.9 kg for adult (Health Canada 1998)

Appendix C. Health Effects Assessment Information

Table C-1: Overview of empirical health effects data availability for the 61 Azo Direct Dyes

C.I. name[a] **CAS RN**	**Cancer bioassay**	**Genetic toxicity**	**Repeated-dose toxicity**	**Acute toxicity**	**Azo bond reduction**
Direct Yellow 11 1325-37-7[b]	–	*In vitro*	–	–	*In vitro*
Direct Yellow 11 lithium salt 65150-80-3[b]	–	See data for Direct Yellow 11	–	–	See data for Direct Yellow 11
Direct Yellow 28 10114-47-3[b]	–	–	–	–	–
71033-21-1[b]	–	–	–	–	–
Direct Green 28 6471-09-6[b]	–	*In vitro*	–	–	*In vitro*
Direct Red 81 triethanolamine salt 75768-93-3[b]	–	*In vivo*; *in vitro*	–	–	–
Direct Violet 51 5489-77-0[b]	–	–	–	–	*In vitro*
Direct Yellow 50 3214-47-9[b]	–	*In vitro*	–	–	*In vitro*
Direct Orange 26 3626-36-6[b]	–	–	–	–	*In vitro*
Direct Red 31 5001-72-9[b]	–	*In vitro*	–	–	*In vitro*
Direct Yellow 34 6420-33-3[b]	–	–	–	–	–
Direct Red 62 6420-43-5	–	–	–	–	–
Direct Orange 72 12217-64-0	–	–	–	–	–
28706-21-0[b]	–	–	–	–	–
38801-08-0	–	–	–	–	–
72152-50-2	–	–	–	–	–
72245-49-9	–	–	–	–	–
72749-87-2	–	–	–	–	–

C.I. name[a] CAS RN	Cancer bioassay	Genetic toxicity	Repeated-dose toxicity	Acute toxicity	Azo bond reduction
72749-88-3	–	–	–	–	–
83221-56-1[b]	–	–	–	–	See data for Direct Orange 26
83221-73-2	–	–	–	–	–
83221-74-3	–	–	–	–	–
83232-28-4	–	–	–	–	–
83232-29-5	–	–	–	–	–
83232-30-8	–	–	–	–	–
83232-31-9	–	–	–	–	–
83232-32-0	–	–	–	–	–
83783-94-2	–	–	–	–	–
83783-95-3	–	–	–	–	–
83783-96-4	–	–	–	–	–
83221-53-8	–	–	–	–	–
83221-54-9	–	–	–	–	–
Direct Red 26 3687-80-7	–	–	–	–	–
Direct Red 4 6420-41-3	–	–	–	–	–
Direct Yellow 26 2829-42-7	–	–	–	–	–
Direct Yellow 12 2870-32-8b	–	*In vivo*; *in vitro*	–	–	*In vitro*
53523-90-3	–	–	–	–	–
72139-21-0	–	–	–	–	–
83783-99-7	–	–	–	–	–
85269-31-4	–	–	–	–	–
110152-63-1	–	–	–	–	–
72869-93-3	–	–	–	–	–
84878-17-1[b]	–	–	–	–	–
84878-16-0	–	–	–	–	–
102082-94-0	–	–	–	–	–
72245-56-8	–	–	–	–	–
Direct Black 56 10134-33-5[b]	–	–	–	–	–
6476-10-4	–	–	–	–	–
10482-42-5	–	–	–	–	–
6406-87-7	–	–	–	–	–
75150-14-0	–	–	–	–	–
71873-49-9	–	–	–	–	–
32829-81-5	–	–	–	–	–
71767-19-6	–	–	–	–	–
83221-68-5	–	–	–	–	–

C.I. name[a] CAS RN	Cancer bioassay	Genetic toxicity	Repeated-dose toxicity	Acute toxicity	Azo bond reduction
83221-69-6	–	–	–	–	–
Direct Blue 71 4399-55-7b	–	*In vitro*	–	–	*In vitro*
Direct Orange 39 1325-54-8b	–	*In vitro*	Oral one-generation study	Oral and dermal acute toxicity studies	*In vitro*
93803-37-3	–	–	–	–	–
85169-18-2	–	–	–	–	–
83221-72-1	–	–	–	–	–

Abbreviations: –, no data; CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index

[a] C.I. names were provided where they are available.

[b] Substances with exposure of the general population of Canada.

Table C-2: Overview of empirical health effects data available for eight Azo Reactive Dyes

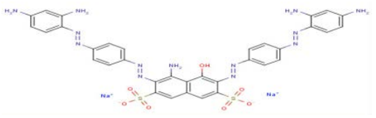
C.I. name[a] CAS RN	Cancer bioassay	Genetic toxicity	Repeated-dose toxicity	Acute toxicity	Azo bond reduction
59641-46-2	–	–	–	–	–
83400-10-6	–	–	–	–	–
Reactive Black 158 83400-11-7[b]	–	–	–	–	–
83400-12-8	–	–	–	–	–
Reactive Black 5 17095-24-8[b]	Inadequate	*In vivo*; *in vitro*	Oral short-term and developmental toxicity studies	Oral acute toxicity studies	*In vivo*; *in vitro*
Reactive Blue 225 108624-00-6[b]	–	–	–	–	–
83399-85-3	–	–	–	–	–
85586-78-3	–	–	–	–	–

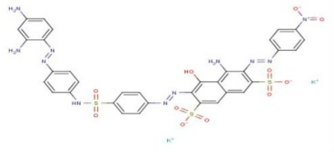
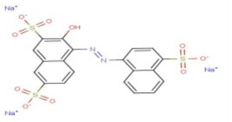
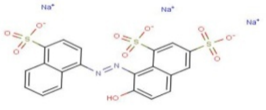
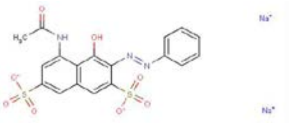
Abbreviations: –, no data; CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index

[a] C.I. names were provided where they are available.

[b] Substances with exposure of the general population of Canada.

Table C-3: Structurally similar substances for the 18 Azo Direct Dyes

Substance name CAS RN	Structure
Direct Black 19 6428-31-5	

Substance name CAS RN	Structure
Acid Black 210 85223-29-6	
An azo direct dye in internal database of Health Canada	Information cannot be disclosed
Amaranth 915-67-3	
New Coccine 2611-82-7	
Acid Red 1 3734-67-6	

Abbreviation: CAS RN, Chemical Abstracts Service Registry Number

Table C-4: Postulated azo bond reductive cleavage products for the 18 Azo Direct Dyes[b]

Parent C.I. name CAS RN[a]	Postulated azo bond reductive cleavage products Name and CAS RN where available or SMILES	Presence of sulfonic acid substituent(s)
Direct Yellow 11 1325-37-7	4-Amino,4'-nitro -2,2'-stilbenedisulfonic acid 119-72-2	Yes
Direct Yellow 11 lithium salt 65150-80-3	4-Amino, 4'-nitro -2,2'-stilbenedisulfonic acid 119-72-2	Yes
Direct Yellow 28 10114-47-3	2-(4-aminophenyl)-6-methylbenzo[*d*]thiazole-7-sulfonic acid C1(c2ccc(N)cc2)=Nc2c(c(S(=O)(=O)O)c(C)cc2)S1	Yes
71033-21-1	2-(4-Aminophenyl)-6-methylbenzothiazole-7-sulfonic acid 130-17-6	Yes
71033-21-1	2-(4-aminophenyl)-6-methylbenzo[*d*]thiazole-5-sulfonic acid Nc1ccc(C2=Nc3c(S2)cc(C)c(S(=O)(O)=O)c3)cc1	Yes
Direct Green 28 6471-09-6	Mesalamine 89-57-6	No
Direct Green 28 6471-09-6	1-amino-4-((4-((4-((4-aminophenyl)amino)-6-(phenylamino)-1,3,5-triazin-2-yl)amino)-3-sulfonatophenyl)amino)-9,10-dioxo-9,10-dihydroanthracene-2-sulfonate, sodium salt (1:2) c12C(=O)c3c(C(=O)c1c(N)c(S(=O)(=O)[O-])cc2Nc1cc(S(=O)(=O)[O-])c(Nc2nc(Nc4ccccc4)nc(Nc4ccc(N)cc4)n2)cc1)cccc3.[Na+].[Na+]	Yes
Direct Red 81	p-Phenylenediamine	No

Parent C.I. name CAS RN[a]	Postulated azo bond reductive cleavage products Name and CAS RN where available or SMILES	Presence of sulfonic acid substituent(s)
triethanolamine salt 75768-93-3	106-50-3	
Direct Red 81 triethanolamine salt 75768-93-3	Sulfanilic acid 121-57-3	Yes
Direct Red 81 triethanolamine salt 75768-93-3	3-amino-7-benzamido-4-hydroxynaphthalene-2-sulfonic acid C(=O)(c1ccccc1)Nc1cc2c(c(O)c(N)c(S(=O)(=O)O)c2)cc1	Yes
Direct Violet 51 5489-77-0	4-Amino-2-methoxy-5-methylaniline 5307-00-6	No
Direct Violet 51 5489-77-0	2-Amino-3,5-xylenesulfonic acid 88-22-2	Yes
Direct Violet 51 5489-77-0	3-amino-4-hydroxy-7-(phenylamino)naphthalene-2-sulfonate, sodium salt (1:1) c1(S(=O)(=O)[O-])c(N)c(O)c2c(cc(Nc3ccccc3)cc2)c1.[Na+]	Yes
Direct Yellow 50 3214-47-9	3-Aminonaphthalene-1,5-disulfonic acid 131-27-1	Yes
Direct Yellow 50 3214-47-9	1,3-bis(4-amino-3-methylphenyl)urea c1(N)c(C)cc(NC(=O)Nc2cc(C)c(N)cc2)cc1	No
Direct Orange 26 3626-36-6	Aniline 62-53-3	No
Direct Orange 26 3626-36-6	7,7'-(carbonylbis(azanediyl))bis(3-amino-4-hydroxynaphthalene-2-sulfonic acid) c1(S(=O)(=O)O)c(N)c(O)c2c(cc(NC(=O)Nc3cc4c(c(O)c(N)c(S(=O)(=O)O)c4)cc3)cc2)c1	Yes
Direct Red 31 5001-72-9	Aniline 62-53-3	No
Direct Red 31 5001-72-9	7,7'-azanediylbis(3-amino-4-hydroxynaphthalene-2-sulfonate), sodium salt (1:2) c1(S(=O)(=O)[O-])c(N)c(O)c2c(cc(Nc3cc4c(c(O)c(N)c(S(=O)(=O)[O-])c4)cc3)cc2)c1.[Na+].[Na+]	Yes
Direct Yellow 34 6420-33-3	3-Aminonaphthalene-1,5-disulfonic acid 131-27-1	Yes
Direct Yellow 34 6420-33-3	1,3-bis(4-amino-2-methoxy-5-methylphenyl)urea c1(NC(=O)Nc2c(OC)cc(N)c(C)c2)c(OC)cc(N)c(C)c1	No
28706-21-0	Amido-G-Acid 86-65-7	Yes
28706-21-0	1,3-bis(4-amino-3-methylphenyl)urea c1(N)c(C)cc(NC(=O)Nc2cc(C)c(N)cc2)cc1	No
83221-56-1	Aniline 62-53-3	No
83221-56-1	7,7'-(carbonylbis(azanediyl))bis(3-amino-4-hydroxynaphthalene-2-sulfonic acid) c1(S(=O)(=O)O)c(N)c(O)c2c(cc(NC(=O)Nc3cc4c(c(O)c(N)c(S(=O)(=O)O)c4)cc3)cc2)c1	Yes
Direct Yellow 12 2870-32-8	p-Phenetidine 156-43-4	No
Direct Yellow 12 2870-32-8	Amsonic acid sodium salt 25394-13-2	Yes

Parent C.I. name CAS RN[a]	Postulated azo bond reductive cleavage products Name and CAS RN where available or SMILES	Presence of sulfonic acid substituent(s)
84878-17-1	4-Nitroaniline 100-01-6	No
84878-17-1	4-amino-1,3-Benzenediol 13066-95-0	No
84878-17-1	3,4,6-triamino-5-hydroxynaphthalene-2,7-disulfonate, potasium salt (1:2) c1(S(=O)(=O)[O-])c(N)c(O)c2c(N)c(N)c(S(=O)(=O)[O-])cc2c1.[K+].[K+]	Yes
84878-17-1	4-amino-*N*-(4-aminophenyl)benzenesulfonamide c1(N)ccc(NS(=O)(=O)c2ccc(N)cc2)cc1	No
Direct Black 56 10134-33-5	Aniline 62-53-3	No
Direct Black 56 10134-33-5	5,8-diaminonaphthalene-2-sulfonate, sodium salt (1:1) c1(N)c2c(c(N)cc1)cc(S(=O)(=O)[O-])cc2.[Na+]	Yes
Direct Black 56 10134-33-5	3,6-diamino-4-hydroxynaphthalene-2-sulfonate, sodium salt (1:1) c1(S(=O)(=O)[O-])c(N)c(O)c2c(ccc(N)c2)c1.[Na+]	Yes
Direct Blue 71 4399-55-7	3-Aminonaphthalene-1,5-disulfonic acid 131-27-1	Yes
Direct Blue 71 4399-55-7	1,4-Naphthalenediamine 2243-61-0	No
Direct Blue 71 4399-55-7	3,7-diamino-4-hydroxynaphthalene-2-sulfonate, sodium salt (1:1) c1(S(=O)(=O)[O-])c(N)c(O)c2c(cc(N)cc2)c1.[Na+]	Yes
Direct Blue 71 4399-55-7	5,8-diaminonaphthalene-2-sulfonate, sodium salt (1:1) c1(N)c2c(c(N)cc1)cc(S(=O)(=O)[O-])cc2.[Na+]	Yes
Direct Orange 39 1325-54-8	p-Phenylenediamine 106-50-3	No
Direct Orange 39 1325-54-8	Sodium sulfanilate 515-74-2	Yes
Direct Orange 39 1325-54-8	4,4'-Diamino-2,2'-stilbenedisulfonic acid 81-11-8	Yes

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index; SMILES, simplified molecular input line entry system

[a] C.I. names were provided where they are available.

[b] Monoazo, Disazo and Polyazo Direct Dyes were separated by double lines.

Table C-5: Overview of carcinogenicity and genotoxicity data for the postulated azo bond reductive cleavage products of the 18 Azo Direct Dyes[a]

Aromatic amine name CAS RN	Parent Azo Direct Dye C.I. name CAS RN	Carcinogenicity	Genotoxicity
4-Nitroaniline 100-01-6	84878-17-1	Negative in rats and female mice; equivocal in male mice	*In vivo,* negative *In vitro,* some positive
p-Phenylenediamine (PPD) 106-50-3	Direct Orange 39 1325-54-8 Direct Red 81 triethanolamine salt 75768-93-3	Negative in rats and mice	*In vivo,* negative *In vitro,* some positive

Aromatic amine name CAS RN	Parent Azo Direct Dye C.I. name CAS RN	Carcinogenicity	Genotoxicity
Sulfanilic acid 121-57-3	Direct Red 81 triethanolamine salt 75768-93-3	–	*In vivo,* – *In vitro*, negative
Sodium sulfanilate 515-74-2	Direct Orange 39 1325-54-8	–	*In vivo,* – *In vitro*, negative
2-(4-Aminophenyl)-6-methylbenzothiazole-7-sulfonic acid 130-17-6	71033-21-1	–	*In vivo,* negative *In vitro*, mixed
p-Phenetidine 156-43-4	Direct Yellow 12 2870-32-8	–	*In vivo,* some positive *In vitro*, some positive
1,4-Naphthylenediamine 2243-61-0	Direct Blue 71 4399-55-7	–	*In vivo,* – *In vitro*, mixed
4-Amino-2-methoxy-5-methylaniline 5307-00-6	Direct Violet 51 5489-77-0	–	*In vivo,* – *In vitro*, negative
Aniline 62-53-3	Direct Orange 26 3626-36-6 Direct Red 31 5001-72-9 Direct Black 56 10134-33-5 83221-56-1	Positive in male rats; negative in female rats and mice	*In vivo,* some positive *In vitro*, some positive
4,4′-Diamino-2,2′-stilbenedisulfonic acid 81-11-8	Direct Orange 39 1325-54-8	Negative in rats and mice	*In vivo,* – *In vitro*, negative
Amido-G-Acid 86-65-7	28706-21-0	Inadequate evidence	*In vivo,* – *In vitro*, negative
Mesalamine 89-57-6	Direct Green 28 6471-09-6	Negative in rats and mice	*In vivo,* negative *In vitro*, negative

Abbreviations: –, no data; CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index

[a] Only the aromatic amines that have empirical data identified are presented.

Table C-6: Postulated azo bond reductive cleavage products for the three Azo Reactive Dyes

Parent substance Name CAS RN[a]	Postulated azo bond reductive cleavage products Name and CAS RN where available or SMILES	Presence of sulfonic acid substituent(s)
Reactive Black 158 83400-11-7	2-amino-5-(((5-chloro-2,6-difluoropyrimidin-4-yl)amino)methyl)naphthalene-1-sulfonate, sodium salt (1:1) c1(N)c(S(=O)(=O)[O-])c2c(c(CNc3c(Cl)c(F)nc(F)n3)ccc2)cc1.[Na+]	Yes
Reactive Black 158 83400-11-7	6-amino-4-benzamido-5-hydroxynaphthalene-1,7-disulfonate, lithium-sodium salt (1:1:1) [Li+].C(=O)(c1ccccc1)Nc1c2c(O)c(N)c(S(=O)(=O)[O-])cc2c(S(=O)(=O)[O-])cc1.[Na+]	Yes
Reactive Black 5 17095-24-8	4-((2-Sulfatoethyl)sulfonyl)aniline 2494-89-5	Yes
Reactive Black 5	3,4,6-triamino-5-hydroxynaphthalene-2,7-disulfonate, sodium	Yes

Parent substance Name CAS RN[a]	Postulated azo bond reductive cleavage products Name and CAS RN where available or SMILES	Presence of sulfonic acid substituent(s)
17095-24-8	salt (1:2) c1(S(=O)(=O)[O-])c(N)c(O)c2c(N)c(N)c(S(=O)(=O)[O-])cc2c1.[Na+].[Na+]	
Reactive Blue 225 108624-00-6	4-((2-Sulfatoethyl)sulfonyl)aniline 2494-89-5	Yes
Reactive Blue 225 108624-00-6	-Amino-4-((5-chloro-2,6-difluoro-4-pyrimidinyl)amino)benzenesulphonic acid 26592-28-9	Yes
Reactive Blue 225 108624-00-6	3,4,6-triamino-5-hydroxynaphthalene-2,7-disulfonate, lithium-sodium salt (1:1:1) [Li+].c1(S(=O)(=O)[O-])c(N)c(O)c2c(N)c(N)c(S(=O)(=O)[O-])cc2c1.[Na+]	Yes

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index; SMILES, simplified molecular input line entry system

Summary C-1: Summary of available data on carcinogenicity and genotoxicity for the postulated azo bond reductive cleavage products of the 18 Azo Direct Dyes

4-Nitroaniline (CAS RN 100-01-6)

The health effects of 4-Nitroaniline were assessed along with Cartain Aromatic Amines in a separate Screening Assessment (Environment Canda and Health Canada 2014b). 4-Nitroaniline did not exhibit carcinogenicity in male and female rats and female mice via 2-year oral (gavage) exposure. It exhibited equivocal evidence of carcinogenicity in male mice (2-year gavage). The incidence of liver hemangiosarcoma and the combined incidence of hemangioma and hemangiosarcoma at all sites were marginally increased at the high dose in male mice. In both cases, a trend test showed significance, but no pairwise comparisons were significant. All *in vivo* genotoxicity assays were negative, including the sex-linked recessive lethal test in *Drosophila*, unscheduled DNA synthesis in rat hepatocytes (gavage) and micronucleus induction in bone marrow of male and female mice (intraperitoneal injection). *In vitro*, 4-nitroaniline was positive for chromosomal aberration in mammalian cells with metabolic activation (S9). Mixed results were observed for chromosomal aberration without S9 and for sister chromatid exchange with and without S9. A forward mutation assay in mouse lymphoma cells was positive without S9 and negative with S9. The results were negative for unscheduled DNA synthesis in primary rat hepatocytes. The majority of Ames assays were negative with and without S9.

p-Phenylenediamine (CAS RN 106-50-3)

p-Phenylenediamine (PPD) was classified by the International Agency for Research on Cancer (IARC 1987) as a Group 3 substance—“Not classifiable as to its carcinogenicity to humans.”

The toxicity of PPD has been reviewed by the Scientific Committee on Consumer Products (SCCP 2006). PPD did not exhibit evidence of carcinogenicity in mice, rats or rabbits following chronic oral or dermal exposure (SCCP 2006). *In vivo*, PPD was negative in various genotoxicity tests, including micronucleus induction in rats and mice via oral gavage or intraperitoneal injection and unscheduled DNA synthesis and the DNA damage assay (comet assay) in rats via oral gavage (SCCP 2006). *In vitro* genotoxicity test results for PPD were mixed. Overall, SCCP (2006) considered that PPD alone is not genotoxic, whereas positive findings were reported from genotoxicity studies when PPD was tested in combination with couplers and/or hydrogen peroxide.

Sulfanilic acid (CAS RN 121-57-3) and sodium sulfanilate (CAS RN 515-74-2)

Sulfanilic acid and its sodium salt are considered toxicologically equivalent. No empirical cancer bioassay or *in vivo* genotoxicity testing data have been identified for these substances. Sulfanilic acid was negative in all available *in vitro* genotoxicity testing, including gene mutation (Ames tests) in various *Salmonella* strains with and without metabolic activation and DNA damage/repair assays (SOS response and DNA strand breaks) in *Escherichia coli* (European Commission ©2000; Ben Mansour et al. 2009).

2-(4-Aminophenyl)-6-methylbenzothiazole-7-sulfonic acid (CAS RN 130-17-6)

No empirical cancer bioassay data for 2-(4-aminophenyl)-6-methylbenzothiazole-7-sulfonic acid have been identified. The REACH dossier for this substance (REACH 2013e) reported some *in vivo* and *in vitro* genotoxicity testing data. This substance did not induce micronucleus formation in mice in a 90-day dietary study. It induced gene mutation in bacteria in one Ames test in *Salmonella* strains TA98 and TA100 with metabolic activation at high dose levels (> 1000 µg/plate), but it was negative in another Ames test in *Salmonella* strains TA98, TA100, TA1535 and TA1537 with and without metabolic activation. The purity of the test material in these studies was not reported.

p-Phenetidine (CAS RN 156-43-4)

The health effects of *p*-Phenetidine were assessed along with Cartain Aromatic Amines in a separate Screening Assessment (Environment Canda and Health Canada 2014a). No empirical cancer bioassay data for *p*-Phenetidine have been identified. *p*-Phenetidine was classified by the European Commission as a Category 2 mutagen—“Suspected of causing genetic defects” (European Commission 2008). *p*-Phenetidine induced micronucleus formation in mice following intraperitoneal injection and *in vitro* gene mutation in *Salmonella* strains TA98 and TA100 with metabolic activation and in mammalian cells with and without metabolic activation (OECD 2002). *p*-Phenetidine also induced chromosomal aberration and DNA strand breaks in mammalian cells *in vitro* (Japan MHLW; Nordernskjöld and Moldéus 1983; Andersson et al. 1982). However, it did not induce mammalian cell transformation (Patierno et al. 1989).

1,4-Naphthalenediamine (CAS RN 2243-61-0)

No empirical cancer bioassay data for 1,4-naphthalenediamine have been identified. For the genetic effects, only two Ames studies were identified for 1,4-naphthalenediamine, and positive results were observed in *Salmonella* strains TA98 and TA100 only in one study with metabolic activation (Mortelmans et al. 1986; Zeiger et al. 1992).

4-Amino-2-methoxy-5-methylaniline (CAS RN 5307-00-6)

No empirical cancer bioassay data for 4-amino-2-methoxy-5-methylaniline have been identified. For the genetic effects, in the only available Ames study, 4-amino-2-methoxy-5-methylaniline was negative in all *Salmonella* strains tested, with and without metabolic activation (Shahin 1994).

Aniline (CAS RN 62-53-3)

The human health risk assessment for aniline was conducted previously by Health Canada (Canada 1994; Health Canada 2011a), and it summarized that “Acute or short-term exposure to aniline has been reported to cause reversible methaemoglobin formation in experimental animals and humans. There is limited evidence of carcinogenicity of aniline in laboratory animals exposed to high doses. The *in vitro* or *in vivo* genotoxicity data were mixed; however, there is no evidence to support the direct genotoxicity potential of aniline” (Health Canada 2011a).

Based on the toxicological data reviewed by Health Canada (Canada 1994; Health Canada 2011a), chronic dietary exposure to aniline has significantly induced splenic tumours in male rats, but not in female rats or mice. *In vivo*, aniline induced DNA damage and micronuclei via oral exposure, but not chromosomal aberration via intraperitoneal injection. *In vitro*, aniline induced chromosomal aberration, but not gene mutation in bacteria (Ames test). Aniline did not induce mammalian cell transformation, and the tests results for micronucleus induction were mixed.

4,4′-Diamino-2,2′-stilbenedisulfonic acid (CAS RN 81-11-8)

4,4′-Diamino-2,2′-stilbenedisulfonic acid did not exhibit evidence of carcinogenicity in rats or mice in 2-year dietary studies (NTP 1992). This substance did not induce gene mutation in bacteria (Ames test) or chromosomal aberration or sister chromatid exchanges in mammalian cells, with and without metabolic activation (Zeiger et al. 1987; Loveday et al. 1990; OECD 2004).

Amido-G-Acid (CAS RN 86-65-7)

The carcinogenic potential of Amido-G-Acid has not been investigated via conventional routes of exposure (oral, dermal or inhalation). In a short-term study, this substance was given to A/St mice via intraperitoneal injection at 325, 625 or 1250 mg/kg-bw per injection, 3 times per week for 5 (high-dose group) to 8 (two lower-dose groups) weeks, and the surviving animals were observed for 24 weeks. Significantly increased incidences of pulmonary adenoma were observed in the lowest dose group only (Theiss et al. 1981). No dose–response relationship was observed. No other health effects were examined. The findings of this study are considered to be inadequate evidence for the potential carcinogenic effects of Amido-G-Acid. Amido-G-Acid was negative in the Ames test (Jung et al. 1992).

Mesalamine (CAS RN 89-57-6)

The health effects of mesalamine were critically reviewed by the US Food and Drug Administration (US FDA 2008). Mesalamine did not exhibit evidence of carcinogenicity in rats or mice in 2-year dietary studies. It did not induce micronucleus formation in mice via oral administration or sister chromatid exchanges in hamsters via intraperitoneal injection. It did not induce gene mutation in bacteria (Ames test), with and without metabolic activation (US FDA 2008).

The remaining postulated azo bond reductive cleavage products

Empirical carcinogenicity and genotoxicity data for 4-amino-1,3-Benzenediol (CAS RN 13066-95-0), 1,3-bis(4-amino-2-methoxy-5-methylphenyl)urea, 1,3-bis(4-amino-3-methylphenyl)urea and 4-amino-*N*-(4-aminophenyl)benzenesulfonamide have not been identified. These substances are not sulfonated, and their carcinogenic and genotoxic potential remains unknown.

4-Amino-4’-nitro-2,2’-stibenedisulfonic acid (CAS RN 119-72-2), 3-aminonaphthalene-1,5-disulfonic acid (CAS RN 131-27-1), Amsonic acid sodium salt (CAS RN 25394-13-2), 2-amino-3,5-xylenesulfonic acid (CAS RN 88-22-2) and the remaining reductive cleavage products are sulfonated aromatic amines. These substances are considered to have low potential to be carcinogenic and genotoxic.

Appendix D. Azo Direct Dyes with Effects of Concern

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

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

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

Substance Names and/or CAS RN	Classification for carcinogenicity[a]	Evidence of carcinogenicity from animal studies and/or human epidemiology	Release of EU22 aromatic amine by azo bond cleavage	Read-across
Direct Red 26 3687-80-7			*o*-Anisidine	
Direct Red 62 6420-43-5			*o*-Toluidine	
72749-87-2			*o*-Toluidine	
72749-88-3			*o*-Anisidine	
83232-30-8			*o*-Toluidine	
83232-32-0			*o*-Toluidine	
84878-16-0			4,4′-thiobisbenzenamine	

[a] Classifications used for carcinogenicity are described in Environment Canada, Health Canada 2014c.