Government of Canada Gouvernement du Canada

Screening Assessment

Aromatic Azo and Benzidine-based Substance Grouping

Certain Azo Solvent Dyes

Environment and Climate Change Canada
Health Canada

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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 22 Azo Solvent Dyes. These substances constitute a subgroup of the Aromatic Azo and Benzidine-based Substance Grouping being assessed as part of the Groupings Initiative of Canada's Chemicals Management Plan (CMP) based on structural similarity and applications. Substances in this grouping were identified as priorities for assessment as they met the categorization criteria under subsection 73(1) of CEPA 1999 and/or were considered as a priority based on other human health concerns.

The Chemical Abstracts Service Registry Number (CAS RN)[1], *Domestic Substances List* (DSL) name, and Colour Index (C.I) name or common name of the 22 substances are presented in the following table.

Identity of the 22 Azo Solvent Dyes in the Aromatic Azo and Benzidine-based Substance Grouping

CAS RN	DSL name	Colour Index name or common name
60-09-3[a]	Benzenamine, 4-(phenylazo)-	Solvent Yellow 1 or *p*-Aminoazobenzene
60-11-7[a]	Benzenamine, *N,N*-dimethyl-4-(phenylazo)-	Solvent Yellow 2
85-83-6[a]	2-Naphthalenol, 1-[[2-methyl-4-[(2-methylphenyl)azo]phenyl]azo]-	Solvent Red 24 or Sudan IV
85-86-9[b]	2-Naphthalenol, 1-[[4-(phenylazo)phenyl]azo]-	Solvent Red 23 or Sudan III
97-56-3[a]	Benzenamine, 2-methyl-4-[(2-methylphenyl)azo]-	Solvent Yellow 3
101-75-7	Benzenamine, *N*-phenyl-4-(phenylazo)-	4-Anilinoazobenzene
103-33-3[a]	Diazene, diphenyl-	Azobenzene
495-54-5	1,3-Benzenediamine, 4-(phenylazo)-	Solvent Orange 3
842-07-9	2-Naphthalenol, 1-(phenylazo)-	Solvent Yellow 14 or Sudan I
1229-55-6[b]	2-Naphthalenol, 1-[(2-methoxyphenyl)azo]-	Solvent Red 1

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

CAS RN	DSL name	Colour Index name or common name
2646-17-5	2-Naphthalenol, 1-[(2-methylphenyl)azo]-	Solvent Orange 2 or Oil Orange SS
2653-64-7	2-Naphthalenol, 1-(1-naphthalenylazo)-	Solvent Red 4
2832-40-8	Acetamide, *N*-[4-[(2-hydroxy-5-methylphenyl)azo]phenyl]-	Solvent Yellow 77[c]
3118-97-6[b]	2-Naphthalenol, 1-[(2,4-dimethylphenyl)azo]-	Solvent Orange 7 or Sudan II
5290-62-0	1-Naphthalenol, 4-[(4-nitrophenyl)azo]-	Magneson II
6368-72-5	2-Naphthalenamine, *N*-ethyl-1-[[4-(phenylazo)phenyl]azo]-	Solvent Red 19
6407-78-9[b]	3*H*-Pyrazol-3-one, 4-[(2,4-dimethylphenyl)azo]-2,4-dihydro-5-methyl-2-phenyl-	Solvent Yellow 18
6535-42-8[b]	1-Naphthalenol, 4-[(4-ethoxyphenyl)azo]-	Solvent Red 3
21519-06-2	3*H*-Pyrazol-3-one, 2,4-dihydro-2-(3-hydroxyphenyl)-5-methyl-4-[[4-(phenylazo)phenyl]azo]-	NA
73507-36-5	2-Naphthalenesulfonic acid, 7-(benzoylamino)-4-hydroxy-3-[[4-[(4-sulfophenyl)azo]phenyl]azo]-, compounds with *N,N*'-bis (mixed Ph and tolyl and xylyl)guanidine monohydrochloride-	NA
73528-78-6	3-Pyridinecarbonitrile, 5-[[4-[(2,6-dichloro-4-nitrophenyl)azo]-2,5-dimethoxyphenyl]azo]-2,6-bis[(2-methoxyethyl)amino]-4-methyl-	NA
85392-21-8	3-Pyridinecarbonitrile, 5-[[2-chloro-4-(phenylazo)phenyl]azo]-2,6-bis[(3-methoxypropyl)amino]-4-methyl-	NA

Abbreviations: 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.

[b] This substance was previously assessed and concluded under the Challenge Initiative of the CMP.

[c] Solvent Yellow 77 is also known as Disperse Yellow 3. The ecological assessment and the section 64 of CEPA 1999conclusions of this substance are deferred to the Azo Disperse Dyes assessment while the human health assessment for this substance is included in this assessment of Azo Solvent Dyes.

Assessments to determine whether five of the Azo Solvent Dyes (Solvent Red 1, Solvent Red 3, Solvent Red 23, Solvent Yellow 18 and Solvent Orange 7) met one or more criteria under section 64 of CEPA 1999 were previously conducted under the Challenge Initiative of the CMP. Among them, one substance (Solvent Red 23) was concluded to meet the criteria set out in paragraph 64*(c)* of CEPA 1999. As outlined in

the *Notice of Intent for the Aromatic Azo and Benzidine-based Substance Grouping*[2], it was recognized that assessments and conclusions pertaining to some of the substances in the grouping may be subsequently updated as part of the current subgroup assessment. Specifically, significant new information has been identified to inform the ecological assessment of the Azo Solvent Dyes subgroup and the assessments for the five substances have been updated accordingly. Similarly, significant new information pertaining to human health has been identified for three of the five substances (Solvent Red 1, Solvent Red 3 and Solvent Yellow 18) and the human health risk assessments for these three substances have been updated.

Solvent Yellow 77 (CAS RN 2832-40-8), also known as Disperse Yellow 3 is included in the Azo Solvent Dyes subgroup, which was established based on the similarity in physical-chemical properties of these substances. However, due to the use of Solvent Yellow 77 in textile dye formulation and textile dyeing reported under section 71 of CEPA 1999, the ecological assessment for this substance is deferred to the Azo Disperse Dyes assessment. However, the human health assessment for this substance, including exposure from its use as a textile dye, is part of the assessment of Azo Solvent Dyes. The section 64 conclusions of CEPA 1999 for this substance are in the Azo Disperse Dyes assessment.

Azo solvent dyes are not expected to occur naturally in the environment. None of the 22 Azo Solvent Dyes were reported to be manufactured in Canada based on recent surveys conducted under section 71 of CEPA 1999; however, five of these substances were reported to be imported into Canada above reporting thresholds (during 2005 or 2008). Some of these substances were also identified as being used in products available to consumers in the Canadian marketplace. No measured concentrations in the Canadian environment have been identified for any of these substances.

Environment

Azo solvent dyes are generally hydrophobic substances that are sparingly soluble in water, with some monoazo substances in this subgroup having experimental water solubilities slightly above 1 mg/L. Given the import and use of five Azo Solvent Dyes in Canada above reporting thresholds, potential releases to the aquatic environment and to the terrestrial environment (via municipal wastewater sludge) have been estimated. When considering potential releases to water, sediment, and soil and the physical and chemical properties of these substances, it is expected that the Azo Solvent Dyes may remain in the water column up to their apparent water solubility limit, and may also

[2] Canada, Dept. of the Environment, Dept. of Health. 2010. *Canadian Environmental Protection Act, 1999*: Notice of intent to assess and manage the risks to the health of Canadians and their environment posed by aromatic azo substances which may break down to certain aromatic amines, substances which may break down to certain benzidines, and the corresponding aromatic amines or benzidines. Canada Gazette, Part I, vol. 144, no. 23, p. 1402–1405. Available from: http://canadagazette.gc.ca/rp-pr/p1/2010/2010-06-05/html/notice-avis-eng.html#d101

ultimately partition to suspended solids, sediments or soil particles. Available experimental and modelled data regarding the abiotic and biotic degradation of the Azo Solvent Dyes indicates that these substances tend to persist in water, sediment and soil. In anaerobic environments (i.e., 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 (K_{ow}) and fish bioconcentration factors (BCFs) indicates that these substances are not likely to bioconcentrate or bioaccumulate in aquatic organisms. These results were substantiated with modelled data that considered metabolism.

All of the structurally related Azo Solvent Dyes (with the exception of CAS RN 73507-36-5) are expected to have a common mode of action with respect to ecotoxicity (based on the reactivity of the amine, aniline, or phenolic functional groups) and thus the toxicity information for aquatic, sediment- and soil-dwelling organisms was applied to all of these 21 structurally-related substances. Toxicity information for these substances indicates that they are hazardous to aquatic organisms at low concentrations. Sediment-dwelling organisms may also be adversely affected, although the available toxicological data are preliminary. Toxicity information for CAS RN 73507-36-5 indicates that it would not be harmful to aquatic organisms at low concentrations.

Aquatic exposure analyses were focussed on scenarios representing potential major environmental releases due to industrial activities involving Azo Solvent Dyes that may result in high levels of exposure to aquatic organisms. Predicted environmental concentrations (PECs) were calculated for the aquatic environment for those substances identified in industrial formulation activities. The PECs did not exceed the predicted no-effect concentration (PNEC) for water.

Considering all available lines of evidence presented in this Screening Assessment, there is low risk of harm to organisms and the broader integrity of the environment from the 21 of 22 substances in the Azo Solvent Dyes subgroup. For the remaining substance, Solvent Yellow 77 (Disperse Yellow 3), the ecological risk is being addressed in the assessment of Azo Disperse Dyes. It is concluded that these 21 Azo Solvent Dyes do not meet the criteria under paragraphs 64(*a*) or 64(*b*) of CEPA 1999, as they are not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

Human Health

With respect to human health, this Screening Assessment addresses 20 of 22 substances in the Azo Solvent Dyes subgroup, including substances previously assessed for which significant new information has become available. The remaining

two substances, Solvent Orange 7 and Solvent Red 23, were previously assessed and concluded under the Challenge Initiative of the CMP. As significant new information relevant to the health assessment was not identified for these two substances, the previous conclusions on human health for these substances have not been updated. However, information on Solvent Orange 7 and Solvent Red 23 was considered to support a read-cross approach for the Sudan Dyes subset in the health assessment.

For the health assessment, the Azo Solvent Dyes were evaluated as part of one of three health subsets; “Azobenzene and Its Derivatives”, “Sudan Dyes” and “Miscellaneous Substances”. Based on the empirical data identified, the critical health effects associated with exposure to Azobenzene and Its Derivatives (i.e., Azobenzene, *p*-Aminoazobenzene, Solvent Yellow 2, Solvent Orange 3, Solvent Yellow 3 and Solvent Yellow 77) are considered to be carcinogenicity and genotoxicity. In addition, Azobenzene, *p*-Aminoazobenzene, Solvent Yellow 2 and Solvent Yellow 77 are considered to have haematological effects. For the Sudan Dyes (i.e., Sudan I, Oil Orange SS, Solvent Red 1 and Sudan IV), based on the empirical data identified and data from read-across, these substances are considered to have carcinogenic and genotoxic potential as well as potential for causing haematological effects. For the Miscellaneous Substances (i.e., Solvent Red 3, Solvent Yellow 18, Solvent Red 19, 4-Anilinoazobenzene, Solvent Red 4, Magneson II, and CAS RN 21519-06-2, CAS RN 73507-36-5, CAS RN 73528-78-6 and CAS RN 85392-21-8), only limited empirical data were identified; hence their critical health effects cannot be conclusively determined.

Exposure for the general population of Canada to the 20 Azo Solvent Dyes through environmental media and food is not expected; therefore risk to human health from these exposures sources is not expected.

Seven Azo Solvent Dyes (Solvent Orange 3, Solvent Yellow 77, Sudan I, Solvent Red 1, Sudan IV, Solvent Red 3 and Solvent Yellow 18) were identified to be used in certain products available to consumers in the Canadian marketplace. Margins between the exposure estimates for Solvent Orange 3, Solvent Yellow 77, Sudan I, Solvent Red 1 and Solvent Red 3 from use of products (shoe polish, textiles, leather, writing ink and cosmetics) containing these substances and the critical health effects levels were considered adequate to address uncertainties in the health effects and exposure databases. Based on available health effects data, Solvent Yellow 18 was not identified as having high hazard potential. Therefore, risk to human health from use of cosmetics containing this dye is considered to be low. Risk to young children who may incidentally ingest paper products containing Solvent Yellow 77 is expected to be low as available information indicates that acute toxicity is not a health concern for this substance. Additionally, exposure to Sudan IV used as a dye in food packaging material is not expected to be significant, therefore risk to human health from this application is considered to be low.

For the remaining 13 Azo Solvent Dyes, no uses of these substances in products available to consumers in the Canadian marketplace were identified. Therefore, based

on available information for exposure in Canada, the risk to human health is not expected for these 13 Azo Solvent Dyes.

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

Based on the information presented in this Screening Assessment, it is concluded that the 19 Azo Solvent Dyes evaluated in this assessment for human health do not meet the criteria under paragraph 64(*c*) of CEPA 1999 as they are not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health. In addition, there are no updates to the conclusions made with respect to paragraph 64*(c)* for Solvent Red 23 and Solvent Orange 7, previously assessed by the Government of Canada under the Challenge Initiative of the CMP. The conclusion with respect to paragraph 64(*c*) of CEPA 1999 for Solvent Yellow 77 (Disperse Yellow 3) is summarized in the Azo Disperse Dyes assessment.

Overall Conclusion

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

While it was determined that CAS RN 2832-40-8 (Solvent Yellow 77, Disperse Yellow 3) did not pose a risk to human health, the section 64 conclusions of CEPA 1999 for this substance are included in the Azo Disperse Dyes assessment.

The conclusion previously made under the Challenge Initiative that Solvent Red 23 meets the criteria set out in paragraph 64*(c)* of CEPA 1999 remains unchanged.

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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 Solvent Dyes subgroup. Consideration of potential azo bond cleavage products (i.e., aromatic amines) is a key element of human health assessment in each subgroup. Some aromatic amines, commonly referred to as EU22 aromatic amines[3], 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 recent document prepared by the Government of Canada (Environment Canada and Health Canada 2013a).

Among the 22 substances in the Azo Solvent Dyes subgroup, 5 substances (Solvent Orange 7, Solvent Red 23, Solvent Red 1, Solvent Red 3 and Solvent Yellow 18) were previously assessed and concluded by the Government of Canada during the Challenge Initiative (Environment Canada and Health Canada 2009, 2010, 2011). Solvent Red 23 was concluded to meet the criteria under paragraph 64(*c*) of CEPA 1999, while the remaining four substances did not meet the criteria. Similarly, four substances (Solvent Red 4, Solvent Red 19, CAS RN 73528-78-6 and CAS RN 85392-21-8) were previously

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

included as part of a screening assessment, in April 2008, of 145 persistent, bioaccumulative, and inherently toxic (PBiT) substances that were considered not to be in commerce (Environment Canada and Health Canada 2008). Certain information on these substances, including submissions pertaining to their uses received at that time, is used here to inform the subgroup assessment of Certain Azo Solvent Dyes (Environment Canada and Health Canada 2009, 2010, 2011). Solvent Yellow 77 (CAS RN 2832-40-8), also known as Disperse Yellow 3 was included in the Azo Solvent Dye subgroup, which was established based on similarity in physical-chemical properties and expected use of these substances (Environment Canada and Health Canada 2013a). However, according to recent surveys conducted under section 71 of CEPA 1999, the substance is primarily used in textile dye formulation and in textile dyeing, which are more in line with azo disperse dye use, therefore, the ecological assessment for this substance is summarized in the Azo Disperse Dyes assessment. However, the human health assessment for this substance , including exposure from its use as a textile dye, is part of the assessment of Azo Solvent Dyes. The section 64 conclusions of CEPA 1999 for this substance are in the Azo Disperse Dyes assessment.

Based on significant new information relevant to the ecological assessment of the Azo Solvent Dyes, 21 substances are assessed with respect to determining risks to the environment. For the human health risk assessment, 20 Azo Solvent Dyes are being assessed, including 3 that were previously assessed and concluded upon (Solvent Red 1, Solvent Red 3 and Solvent Yellow 18), as significant new information relevant to human health exposure has been identified for these 3 substances.

Screening assessments focus on information critical to determining whether substances meet the criteria as set out in section 64 of CEPA 1999, by examining scientific information to develop conclusions by incorporating a weight of evidence approach and precaution[4].

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 March 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.

[4] 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 *Hazardous Products Regulations* and the *Controlled Products Regulations*, which are 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.

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 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.

2. Identity of Substances

The nomenclature and chemical structure descriptions of the 22 Azo Solvent Dyes are presented below. These substances have been organized into structurally related eco-subsets and health subsets to inform the ecological and human health assessments of these substances, respectively, in considering overall data availability and the potential to share data for read-across purposes. Structurally related subsets formed for the ecological assessment consist of substances that are considered structural analogues of each other, given their overall chemical similarity and common functional groups (eco-subsets A through H, with increasing molecular weight across the subsets). For the human health assessment, subsets were identified by considering structural similarities and modes of action with respect to their health effects. These general approaches are discussed further in the sections on Selection of Analogues for Read-across and Use of (Q)SAR Models, Environmental Persistence and the Potential to Cause Harm to Human Health.

The identities of the individual substances in this Screening Assessment are presented in Table 2-1, with descriptions of the ecological (eco) and human health (health) structural and functional groups presented in Table 2-2. Colour Index (C.I.) names and/or common names of the substances are used in this report, where available, to better inform the readers. A list of additional chemical names (e.g., trade names) is available from the National Chemical Inventories (NCI 2012). Simplified molecular input line entry system (SMILES) strings for these substances are presented in Environment Canada (2013a).

Table 2-1. Identity of Azo Solvent Dyes and division into subsets for ecological and human health assessments

CAS RN	DSL name	C.I. name and/or common name	Eco-subset	Health subset
60-09-3	Ben zenamine, 4-(phenylazo)-	Solvent Yellow 1 or *p*-aminoazobenzene	A	Azobenzene and Its Derivatives
60-11-7	Benzenamine, *N*,*N*-dimethyl-4-(phenylazo)-	Solvent Yellow 2		
97-56-3	Benzenamine, 2-methyl-4-[(2-methylphenyl)azo]-	Solvent Yellow 3		
103-33-3	Diazene, diphenyl-	Azobenzene		
495-54-5	1,3-Benzenediamine, 4-(phenylazo)-	Solvent Orange 3		
2832-40-8	Acetamide, *N*-[4-[(2-hydroxy-5-methylphenyl)azo]phenyl]-	Solvent Yellow 77 or Disperse Yellow 3		
101-75-7	Benzenamine, *N*-phenyl-4-(phenylazo)-	4-Anilinoazobenzene	B	Miscellaneous Substances
842-07-9	2-Naphthalenol, 1-(phenylazo)-	Solvent Yellow 14 or Sudan I or Disperse Yellow 97	C	Sudan Dyes
1229-55-6[b]	2-Naphthalenol, 1-[(2-methoxyphenyl)azo]-	Solvent Red 1		

CAS RN	DSL name	C.I. name and/or common name	Eco-subset	Health subset
2646-17-5	2-Naphthalenol, 1-[(2-methylphenyl)azo]-	Solvent Orange 2 or Oil Orange SS		
3118-97-6[a]	2-Naphthalenol, 1-[(2,4-dimethylphenyl)azo]-	Solvent Orange 7 or Sudan II		
5290-62-0	1-Naphthalenol, 4-[(4-nitrophenyl)azo]-	Magneson II		Miscellaneous Substances
6535-42-8[b]	1-Naphthalenol, 4-[(4-ethoxyphenyl)azo]-	Solvent Red 3		
2653-64-7[c]	2-Naphthalenol, 1-(1-naphthalenylazo)-	Solvent Red 4		
6407-78-9[b]	3H-Pyrazol-3-one, 4-[(2,4-dimethylphenyl)azo]-2,4-dihydro-5-methyl-2-phenyl-	Solvent Yellow 18	D	
85-83-6	2-Naphthalenol, 1-[[2-methyl-4-[(2-methylphenyl)azo]phenyl]azo]-	Solvent Red 24 or Sudan IV	E	Sudan Dyes
85-86-9[a]	2-Naphthalenol, 1-[[4-(phenylazo)phenyl]azo]-	Solvent Red 23 or Sudan III		
6368-72-5[c]	2-Naphthalenamine, *N*-ethyl-1-[[4-(phenylazo)phenyl]azo]-	Solvent Red 19		Miscellaneous Substances
21519-06-2	3H-Pyrazol-3-one, 2,4-dihydro-2-(3-hydroxyphenyl)-5-methyl-4-[[4-(phenylazo)phenyl]azo]-	NA	F	
73528-78-6[c]	3-Pyridinecarbonitrile, 5-[[4-[(2,6-dichloro-4-nitrophenyl)azo]-2,5-dimethoxyphenyl]azo]-2,6-bis[(2-methoxyethyl)amino]-4-methyl-	NA	G	
85392-21-8[c]	3-Pyridinecarbonitrile, 5-[[2-chloro-4-(phenylazo)phenyl]azo]-2,6-bis[(3-methoxypropyl)amino]-4-methyl-	NA		
73507-36-5	2-Naphthalenesulfonic acid, 7-(benzoylamino)-4-hydroxy-3-[[4-[(4-sulfophenyl)azo]phenyl]azo]-, compounds with *N*,*N*′-bis (mixed Ph and tolyl and xylyl) guanidine monohydrochloride-	NA (UVCB)	H	

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; DSL, Domestic Substances List; C.I., Colour Index; NA, not available; Ph, phenyl; UVCB, unknown or variable composition, complex reaction products, or biological materials

[a] Solvent Red 23 and Solvent Orange 7 were previously assessed and concluded under the Challenge Initiative of the CMP. Solvent Red 23 was concluded to meet the criteria of paragraph 64(c) under CEPA 1999 while Solvent Orange 7 was concluded to not meet this criteria. No significant new information related to the health assessment were identified for these substances, therefore the previous conclusions on human health for Solvent Red 23 and Solvent Orange 7 have not been updated in this Screening Assessment. However, significant new information on ecological risk of both these substances were identified since the previous assessment under the Challenge Initiative, therefore both Solvent Red 23 and Solvent Orange 7 are considered for ecological risk in this Screening Assessment.

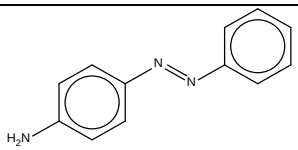
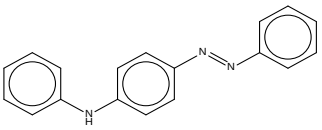
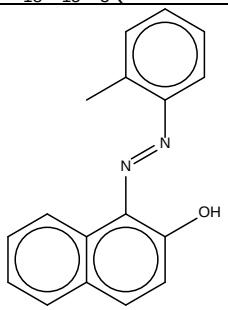
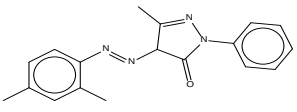
[b] These substance were previously assessed and concluded under the Challenge Initiative of the CMP. Significant new information were identified for these substances and therefore the conclusions were updated for both the human health and ecological risk in this Screening Assessment.

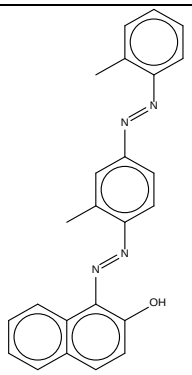
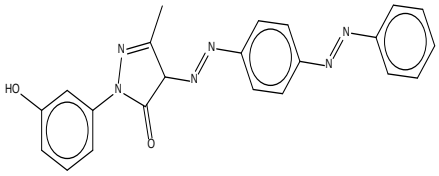
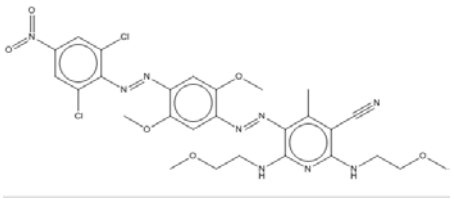
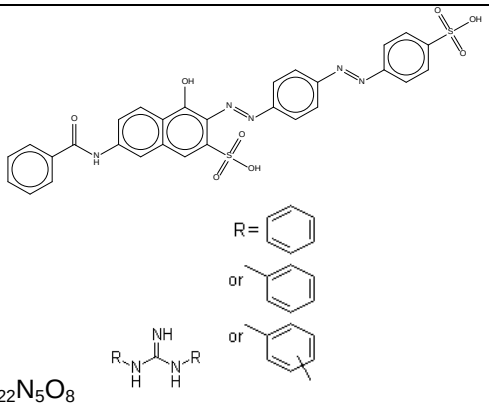
[c] These substances were previously included as part of a screening assessment, in April 2008, of 145 persistent, bioaccumulative, and inherently toxic (PBiT) substances that were considered not to be in commerce. Significant new information were identified for these substances and therefore the conclusions were updated for both the human health and ecological risk in this Screening Assessment.

The same CAS RN can be associated with one or more C.I. names, indicating that the colourant is associated with a different application class or use. The C.I. names Solvent Yellow 77, Solvent Yellow 92 and Disperse Yellow 3 are all associated with the same CAS RN (2832-40-8). Other CAS RNs are also associated with more than one C.I. name, such as Solvent Yellow 14 and Disperse Yellow 97 (CAS RN 842-07-9) and Solvent Red 4 and Pigment Red 40 (CAS RN 2653-64-7). Sudan dyes are often referred to as Sudan I, II, III and IV in the toxicological literature (mainly with respect to human health), but are also known by their C.I. names of Solvent Yellow 14, Solvent Orange 7, Solvent Red 23 and Solvent Red 24, respectively.

A representative substance for each of the structurally related subsets identified for the ecological assessment is presented in Table 2-2. The chemical structure and molecular formula of the representative substance as well as the critical functional groups and molecular weight range (rounded to the nearest gram per mole) of the subset are also indicated. Such information is listed for each of the individual Azo Solvent Dyes in Appendix A (Tables A-1 and A-2).

Table 2-2. Example structures and structural descriptions for Azo Solvent Dyes

Structurally similar eco-subset[a]	Example structure, molecular formula and C.I. name, common name or CAS RN	Critical functional groups	Molecular weight range (g/mol)
A (*n* = 6)	 $C_{12}H_{11}N_3$ (*p*-Aminoazobenzene)	2 benzene rings; methyl, hydroxyl, amine and/or ketone groups; monoazo	182–269
B (*n* = 1)	 $C_{18}H_{15}N_3$ (4-Anilinoazobenzene)	3 benzene rings; amine group; monoazo	273
C (*n* = 7)	 $C_{17}H_{14}N_2O$ (Oil Orange SS)	1 benzene ring and 1 set of naphthalene rings; methyl, hydroxyl, methoxy, ether and/or nitro groups; monoazo	248–298
D (*n* = 1)	 $C_{18}H_{18}N_4O$ (Solvent Yellow 18)	2 benzene rings and substituted pyrazolone ring; methyl and ketone groups; monoazo	306

Structurally similar eco-subset[a]	Example structure, molecular formula and C.I. name, common name or CAS RN	Critical functional groups	Molecular weight range (g/mol)
E (*n* = 3)	 $C_{24}H_{20}N_4O$ (Sudan IV)	2 benzene rings and 1 set of naphthalene rings; hydroxyl, methyl and/or amine groups; disazo	352–380
F (*n* = 1)	 $C_{22}H_{18}N_6O_2$ CAS RN 21519-06-2	3 benzene rings and 1 substituted pyrazolone ring; hydroxyl, ketone and methyl groups; disazo	398
G (*n* = 2)	 $C_{27}H_{29}Cl_2N_9O_6$ CAS RN 85392-21-8	3 substituted benzene rings; methyl, ether, nitro, nitrile and/or chloro groups; disazo	535–646
H (*n* = 1)	 $C_{29}H_{22}N_5O_8$ CAS RN 73507-36-5 (UVCB)	3 benzene rings and 1 set of naphthalene rings; sulfonic acid, hydroxyl, carboxamide groups; disazo; with mixed phenyl, tolyl and xylyl guanidine monohydrochloride	569

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; UVCB, unknown or variable composition, complex reaction products, or biological materials

[a] *n* = number of substances in the eco-subset.

The monoazo solvent dyes (eco-subsets A–D) have one azo bond and a molecular weight of 182–306 g/mol. The disazo solvent dyes (eco-subsets E–H) have two azo bonds and a molecular weight of 352–646 g/mol. The majority of these substances contain 2–4 benzene rings each, with substances in structurally related eco-subsets C and E consisting of one set of naphthalene rings and substances in structurally related eco-subsets D and F having one pyrazolone ring. The two substances in eco-subset G are the only chlorinated substances among these Azo Solvent Dyes. A variety of other functional groups are associated with the Azo Solvent Dyes, and they can influence their physical-chemical properties, environmental fate and toxicological behaviour.

The disazo substance in eco-subset H is a UVCB (unknown or variable composition, complex reaction products, or biological materials) substance that may be characterized by more than one structure in differing proportions. Although the composition is not explicitly known, it may include Solvent Red 33 (CAS RN 61813-61-4) and/or a substance (CAS RN 25188-42-5) that is similar to the neutral form of Direct Red 81, which contains two sulfonic acid groups. These substances may be associated with *N*,*N*′-bis (phenyl, tolyl and xylyl) guanidine monohydrochloride. Sulfonic acid groups are an uncharacteristic component of solvent dyes in general; however, dyes soluble in alcohol solvents include salts formed between sulfonated dyes (usually azo dyes or phthalocyanines) and organic bases of high molecular weight (e.g., diarylguanidine) (CII 2011). No other information is available with which to comprehensively characterize this substance.

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 is provided in Environment Canada and Health Canada (2013a). For characterization of human health effects, the basis for the use of analogues and/or (Q)SAR modelling data is documented in the Health Effects Assessment section of this report.

Analogues used to inform the ecological assessment were selected based on structural similarity and the availability of relevant empirical data pertaining to physical-chemical properties, persistence, bioaccumulation and ecotoxicity. Such data were used as read-across data for those Azo Solvent 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 Solvent Dyes is determined on a case-by-case basis.

A list of the various analogues used to inform this assessment is presented in Table 2-3, along with an indication of the potential read-across data available for different

parameters. All of these substances are azo compounds, most of them being azo solvent or azo disperse dyes. More detailed information regarding the identity of these substances can be found in Appendix A (Table A-3).

Table 2-3. Analogues used to inform various parameters evaluated in this assessment and the availability of potential read-across data for these parameters

CAS RN	C.I. name or common name (eco-subset or health subset)	Physical-chemical data	Fate data	Ecotoxicity data	Human health data
85-84-7	Solvent Yellow 5 or Oil Yellow AB (eco subset C)	X	NA	X	N/A
131-79-3	Solvent Yellow 6 or Oil Yellow OB (eco subset C)	X	NA	X	N/A
532-82-1	Basic Orange 2 (health subset: Azobenzene and Derivatives – Solvent Orange 3)	N/A	N/A	N/A	X
1689-82-3	Solvent Yellow 7 (eco subset A)	X	NA	X	N/A
2610-11-9	Direct Red 81 (eco subset H)	NA	NA	X	N/A
4314-14-1	Solvent Yellow 16 or Sudan Yellow 3G (health subset: Miscellaneous – Solvent Yellow 18)	N/A	N/A	N/A	X
40690-89-9	Disperse Orange 73 (eco subset E)	NA	X	N/A	N/A
61968-52-3	Disperse Red 167 (eco subset G)	NA	X	N/A	N/A
71767-67-4	Disperse Yellow 163 (eco subset G)	NA	X	N/A	N/A

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

Details of the read-across data available for the analogues chosen to inform the ecological and human health assessments of the Azo Solvent Dyes are further discussed in the relevant sections of this report.

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 types of application. Such properties also play a critical role in determining the environmental fate of substances (including their potential for long-range transport), as well as their toxicity to humans and non-human organisms.

A summary of experimental physical and chemical properties for the Azo Solvent Dyes is presented in Table 3-1and Table 3-2. The physical state of those substances for which this information is available is a powder or crystalline powder form. The range of values for each property is indicated for each eco-subset. Detailed substance-specific information for the individual Azo Solvent Dyes can be found in Appendix B (Tables B-1 and B-2) and in Environment Canada (2013a) (e.g., maximum cross-sectional diameters or $D_{min\text{-}max}$, average effective cross-sectional diameters or D_{eff}, and modelled pK_a values). Read-across data from structural analogues used in the ecological assessment are also summarized in Appendix B (Table B-3). Where no information was available for a particular property, analogue data were used or (Q)SAR models were used to predict these values for the neutral form of the substance (Appendix B, Table B-4). It was deemed appropriate to use (Q)SAR models to predict the physical and chemical properties of the monoazo solvent dyes, given that their structures are relatively simple and they have some degree of solubility. (Q)SAR models have not been used to predict the physical and chemical properties of the disazo solvent dyes, which may act more like particles due to their low water solubility. (Q)SAR models were not used for CAS RN 73507-36-5 (the UVCB), which has ionizable sulfonic acid groups (ionizing substances are not in the model domain). A comparison of the modelled values with available experimental values for the monoazo solvent dyes indicates that the (Q)SAR models predict these parameters quite well in most cases, which may indicate that these substances exist as single molecules (Tables B-1 and B-4).

Table 3-1. Range of experimental and predicted physical and chemical properties (at standard temperature) for the monoazo solvent dyes

Eco-subset	Property	Value or range	Type of data
A (*n* = 6)	Melting point (°C)	68–195	Experimental
	Vapour pressure (Pa)	6.67×10^{-9} – 0.048	Experimental
		6.35×10^{-8} – 0	Modelled
	Henry's Law constant ($Pa \cdot m^3/mol$)	1.96×10^{-10} – 1.49	Modelled
	Water solubility (mg/L)	<0.1–34	Experimental
	Log K_{ow} (dimensionless)	1.5–4.6	Experimental
	Log K_{oc} (dimensionless)	2.5–3.8	Modelled
	D_{eff} (nm)	0.69–0.84	Calculated
	$D_{min\text{-}max}$ (nm)	1.06–1.69	Calculated
	pK_a (dimensionless)	pK_{a1} = −0.2 to 2.9 pK_{a2} = 4.9 to 9.5	Modelled
B (*n* = 1)	Melting point (°C)	89–91	Experimental
	Vapour pressure (Pa)	1.87×10^{-4}	Modelled
	Henry's Law constant ($Pa \cdot m^3/mol$)	2.91×10^{-4}	Modelled

Eco-subset	Property	Value or range	Type of data
	Water solubility (mg/L)	< 0.1	Experimental
	Log K_{ow} (dimensionless)	5.4	Modelled
	Log K_{oc} (dimensionless)	4.3	Modelled
	D_{eff} (nm)	0.77	Calculated
	$D_{min\text{-}max}$ (nm)	1.72–1.81	Calculated
	pK_a (dimensionless)	pK_{a1} = −0.8	Modelled
C (*n* = 7)	Melting point (°C)	131–270	Experimental
	Vapour pressure (Pa)	5.01×10^{-8}	Experimental
		$3.56 \times 10^{-8} – 1.27 \times 10^{-5}$	Modelled
	Henry's Law constant (Pa·m^3/mol)	$5.97 \times 10^{-8} – 1.84 \times 10^{-5}$	Modelled
	Water solubility (mg/L)	$3.3 \times 10^{-4} – 8$	Experimental
	Log K_{ow} (dimensionless)	7.5	Experimental
		5.2–6.7	Modelled
	Log K_{oc} (dimensionless)	4.6–5.2	Modelled
	D_{eff} (nm)	0.89–0.95	Calculated
	$D_{min\text{-}max}$ (nm)	1.12–1.74	Calculated
	pK_a (dimensionless)	pK_{a1} = 7.7 pK_{a2} = 8.9	Modelled
D (*n* = 1)	Melting point (°C)	197	Modelled
	Vapour pressure (Pa)	4.23×10^{-7}	Modelled
	Henry's Law constant (Pa·m^3/mol)	1.76×10^{-4}	Modelled
	Water solubility (mg/L)	6.29×10^{-2}	Modelled
	Log K_{ow} (dimensionless)	5.7	Modelled
	Log K_{oc} (dimensionless)	4.5	Modelled
	D_{eff} (nm)	0.97	Calculated
	$D_{min\text{-}max}$ (nm)	1.33–1.76	Calculated
	pK_a (dimensionless)	pK_{a1} = 1.6 pK_{a2} = 8.6	Modelled

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

Table 3-2. Range of experimental physical and chemical properties (at standard temperature) for the disazo solvent dyes

Eco-subset	Property	Value or range	Type of data
E (*n* = 3)	Melting point (°C)	130–195	Experimental
	Vapour pressure (Pa)	NA	NA
	Henry's Law constant (Pa·m^3/mol)	NA	NA
	Water solubility (mg/L)	< 0.0137–0.7	Experimental
	Log K_{ow} (dimensionless)	NA	NA
	Log K_{oc} (dimensionless)	NA	NA
	D_{eff} (nm)	0.95–1.10	Calculated
	$D_{min\text{-}max}$ (nm)	1.26–2.04	Calculated
	pK_a (dimensionless)	pK_{a1} = 3.7–8.9	Modelled
F (*n* = 1)	Melting point (°C)	NA	NA
	Vapour pressure (Pa)	NA	NA
	Henry's Law constant (Pa·m^3/mol)	NA	NA
	Water solubility (mg/L)	NA	NA
	Log K_{ow} (dimensionless)	NA	NA
	Log K_{oc} (dimensionless)	NA	NA
	D_{eff} (nm)	1.02	Calculated
	$D_{min\text{-}max}$ (nm)	1.57–2.36	Calculated

Eco-subset	Property	Value or range	Type of data
	pK_a (dimensionless)	pK_{a1} = 1.6 pK_{a2} = 8.2 pK_{a3} = 10.5	Modelled
G (*n* = 2)	Melting point (°C)	NA	NA
	Vapour pressure (Pa)	NA	NA
	Henry's Law constant (Pa·m^3/mol)	NA	NA
	Water solubility (mg/L)	NA	NA
	Log K_{ow} (dimensionless)	NA	NA
	Log K_{oc} (dimensionless)	NA	NA
	D_{eff} (nm)	1.30–1.33	Calculated
	$D_{min-max}$ (nm)	1.59–2.68	Calculated
	pK_a (dimensionless)	pK_{a1} = 0.8–0.9	Modelled
H (*n* = 1)	Melting point (°C)	NA	NA
	Vapour pressure (Pa)	NA	NA
	Henry's Law constant (Pa·m^3/mol)	NA	NA
	Water solubility (mg/L)	NA	NA
	Log K_{ow} (dimensionless)	NA	NA
	Log K_{oc} (dimensionless)	NA	NA
	D_{eff} (nm)	1.19	Calculated
	$D_{min-max}$ (nm)	1.49–2.99	Calculated
	pK_a (dimensionless)	pK_{a1} = −1.5 pK_{a2} = 1.6	Modelled

Abbreviations: D_{eff}, average effective cross-sectional diameter; $D_{min-max}$, range of maximum cross-sectional diameters; K_{ow}, octanol–water partition coefficient; K_{oc}, organic carbon–water partition coefficient; NA, not available; pK_a, acid dissociation constant

Some of the Azo Solvent Dyes are weakly ionizable, given their functional groups (e.g., phenols). The results of the ACD/pKa GALAS model (ACD/Percepta ©1997–2012) to predict the pK_a values and ionization patterns of these substances are summarized in Environment Canada (2013a). All of these substances, including those that are weakly ionizable, would be found in the neutral form at pH 6–8, whereas above pH 8, many of the substances would have an increasingly significant proportion (> 50%) as a net negative charge. The exception may be CAS RN 73507-36-5 (the UVCB), which would have a net negative charge above pH 6, as it would be expected to dissociate in the aquatic environment, liberating the guanidine counterions. Some of the Azo Solvent Dyes have free amine groups that would be protonated at lower pH and therefore may have corresponding salts of the cation (e.g., Solvent Orange 3 versus Basic Orange 2).

Solvent dyes are described as sparingly soluble in water (< 100 mg/L; Environment Canada and Health Canada 2013a), but typically soluble in a variety of solvents. This characteristic enables them to be dissolved in their substrates (e.g., oil, fats and waxes; alcohol solvents; ethers, esters and ketones; and hydrocarbons, including chlorinated ones) (CII 2011) and used in a variety of applications, as described further in the Uses section. The eco-subsets A and C have some substances with water solubilities above 1 mg/L, whereas substances in other groups have water solubilities below or much below 1 mg/L and are practically insoluble. One of the possible components of CAS RN 73507-36-5 has two sulfonic acid groups that would be expected to make it more water soluble.

The limited experimental data for a few monoazo solvent dyes indicate that generally these substances have low vapour pressures and low Henry's Law constants. Modelled vapour pressures and Henry's Law constants for the remaining substances decrease with increasing molecular weight. Based on the literature, it is expected that the Azo Solvent Dyes would have very low vapour pressures and very low Henry's Law constants (Baughman and Perenich 1988b; Øllgaard et al. 1998).

Cross-sectional diameters of molecules are important in determining their ability to cross biological membranes (as discussed in the Potential for Bioaccumulation section). The average effective cross-sectional diameters for the monoazo and disazo solvent dyes range from 0.69 to 0.97 nm and from 0.95 to 1.33 nm, respectively, and were calculated using CPOPs (2012). The range in maximum cross-sectional diameters for the monoazo and disazo solvent dyes range from 1.06 to 1.81 nm and from 1.26 to 2.99 nm, respectively. The cross-sectional diameters generally increase with increasing molecular weight (Environment Canada 2014).

Similar to other azo colourants, Azo Solvent Dyes may undergo tautomerization between the azo and hydrazone forms. This tautomerization is well known for the Azo Solvent Dyes, where a hydroxyl or amine group and the azo bond are present in the *ortho* position. Tautomerization is important commercially, since the tautomeric forms may differ in colour, performance properties, toxicological profile and tinctorial strength (Environment Canada and Health Canada 2013a). However, the degree to which this affects the fate and behaviour of these substances in the environment or the toxicological properties as they relate to human health or the health of non-human organisms is not well understood.

4. Sources and Uses

4.1 Sources

All of the Azo Solvent Dyes are synthetic dyes and therefore do not occur naturally in the environment.

In recent years (2005 to present), all substances included in this Screening Assessment have been included in surveys issued pursuant to section 71 of CEPA 1999. Nine substances were included in a survey for the 2005 calendar year (Canada 2006), five of those substances were also included in surveys for the 2006 calendar year as part of the Challenge Initiative (Canada 2008a, b), seven substances were included in Phase One of the *Domestic Substances List* (DSL) Inventory Update survey (Canada 2009) and six substances were included in a survey for the 2010 calendar year that focused on the Aromatic Azo and Benzidine-based Substance Grouping (Canada 2011).

None of the Azo Solvent Dyes are known to be manufactured above the reporting thresholds in Canada. In the above-mentioned surveys, only five of the substances were reported as being imported into Canada above the reporting threshold for the reporting year: Solvent Yellow 77 (eco-subset A); Sudan I, Oil Orange SS and Solvent Red 3 (eco-subset C); and Solvent Red 23 (eco-subset E). Two of these five substances (Solvent Red 3 and Solvent Red 23) were previously assessed in the Challenge. Total quantity imported for Solvent Yellow 77, Sudan I and Oil Orange SS for the 2008 reporting year was between 1000 and 10 000 kg according to information submitted in response to Phase 1 of the DSL Inventory Update (Canada 2009; Environment Canada 2009). Total quantities imported for Solvent Red 23 and Solvent Red 3 for the 2005 reporting year were 1000–10 000 kg. A number of these five substances had declarations of stakeholder interest.

In 2005, four companies each reported importing 100–1000 kg of Solvent Red 23 (Environment Canada 2006). Eight companies identified themselves as having a stakeholder interest in Solvent Red 23 in that year. Nine companies reported a stakeholder interest in 2006; in more than one case, the interest involved import or current use of the substance in Canada below the threshold (Environment Canada 2008).

One company reported importing between 100 and 1000 kg of Solvent Red 3 into Canada in response to a CEPA 1999 section 71 survey notice for the 2005 calendar year (Environment Canada 2006). Six companies reported a stakeholder interest in this substance.

4.2 Uses

In general, Azo Solvent Dyes are used principally in lacquers and varnishes, printing inks, stains and plastics (Ishikawa et al. 2008; Kirk-Othmer 2010). They are also used to colour cosmetics, waxes (e.g., candles), soaps, fats, oils and gasoline. Some of these substances were reported to be used as dyes or as intermediates in dyes in the textile, leather and paper industries (Tincher and Robertson 1982; HSDB 1983–; IARC 1990; ETAD 1994, 1995; RAPEX 2012; Scorecard 2011).

Table 4-1 presents a summary of the major uses of the Azo Solvent Dyes in Canada based on Consumer and Commercial Codes submitted in response to Phase 1 of the DSL Inventory Update survey (Canada 2009; Environment Canada 2009); some reported uses are not included in Table 4-1 due to confidentiality. Information regarding uses of Solvent Red 23 may be found in a previous Screening Assessment report (Environment Canada and Health Canada 2011).

Table 4-1. Summary of the major uses of the Azo Solvent Dyes in Canada based on reported Consumer and Commercial Codes submitted in response to the DSL Inventory Update survey (Canada 2009; Environment Canada 2009)

Azo Solvent Dye	Lawn and garden care (C407)[a]	Fuels and related products, mixtures or manufactured items (C404)[a]	Ink, toner and colourants (C306)[a]	Reporting Year
Solvent Yellow 77			X	2008
Sudan I	X	X		2008

[a] Consumer and Commercial Codes are indicated in parentheses.

In Canada, food colouring agents are regulated as food additives under the *Food and Drug Regulations*. Colours that are permitted for use in food are listed in the *List of Permitted Colouring Agents* incorporated by reference in the *Marketing Authorization for Food Additives That May Be Used as Colouring Agents*, issued under the authority of the *Food and Drugs Act*. None of the 22 substances in this Screening Assessment are listed on the *List of Permitted Colouring Agents* as permitted food colouring agents. However, Sudan I may be considered a subsidiary dye of Sunset Yellow (E110) if found at very low levels (2011 email from the Food Directorate, Health Canada, to the Risk Management Bureau, Health Canada; unreferenced).

One substance, Sudan IV, was identified for use in food packaging applications in Canada as a component of dyes that may be used in resins to package food (2011 emails from the Food Directorate, Health Canada, to the Risk Management Bureau, Health Canada; unreferenced).

Colouring agents that are permitted to be used in drugs in Canada are regulated under Part C, Division 1, of the *Food and Drug Regulations* (Canada [1978]). Sudan IV was identified in the Drug Products Database as an active ingredient in veterinary drugs (DPD 2012; 2011 email from the Veterinary Drugs Directorate, Health Canada, to the

Risk Management Bureau, Health Canada; unreferenced). None of the 22 substances in this Screening Assessment have been identified as being used in biologics in Canada (2011 email from the Biologics and Genetic Therapies Directorate, Health Canada, to the Risk Management Bureau, Health Canada; unreferenced).

Sudan IV is listed in the NHPID and is classified as a non-natural health product ingredient because it is not a naturally occurring substance included in Schedule 1 of the *Natural Health Products Regulations* (NHPID 2011) and is not listed in the LNHPD to be present in currently licensed natural health products (LNHPD 2008).

Based on notifications submitted under the *Cosmetic Regulations* to Health Canada, Solvent Red 1, Solvent Red 3 and Solvent Yellow 18 are used as ingredients in certain cosmetic products in Canada, such as hair products, soaps, bath products, fragrances, massage oils and hair removal products (2011 and 2013 emails from the Consumer Product Safety Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada; unreferenced). Cosmetic products containing these substances were not identified in previous Screening Assessment reports (Environment Canada and Health Canada 2009, 2010). Solvent Red 1 was identified as an ingredient in lipstick and cosmetic tattoo ink products (Lundsgaard 2002; Danish EPA 2012b); however, such products containing this substance that were previously notified to Health Canada are no longer available on the Canadian market (2013 email from the Consumer Product Safety Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada; unreferenced).

Solvent Orange 7 and Sudan IV are listed under the names CI 12140 and Solvent Red 24, respectively, 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 products containing certain substances are unlikely to be classified as a cosmetic under the *Food and Drugs Act* (FDA), and in addition,that certain substances, when present in a cosmetic, may contravene (a) the general prohibition found in section 16 of the *Food and Drugs Act* or (b) a provision of the *Cosmetic Regulations* (Health Canada 2014). Solvent Red 23 was added to the Cosmetic Ingredient Hotlist with specific restrictions on use conditions after its screening assessment; details of the restrictions are found in the Cosmetic Ingredient Hotlist (Health Canada 2014).

The use of Solvent Yellow 3 as an adhesive in military applications in Canada was identified (2011 email from the Department of National Defence to the Risk Management Bureau, Health Canada; unreferenced).

Sudan I was also reported to be contained in approximately 70 children's products, including plastic toys, clothing and textile furnishings, on the American market according to data submitted to the Washington State Department of Ecology from October 2011 to July 2014 under the Children's Safe Products Act (Washington State Department of Ecology 2014). Sudan I is also used in ballpoint pen inks, based on information in the

jointly published Colour Index International database of the Society of Dyers and Colourists and the American Association of Textile Chemists and Colorists (CII 2011).

Solvent Orange 3 was identified as an ingredient in 2 common shoe polish products in the United States (HPDB 2012), which are also available on the Canadian market.

5. Environmental Fate and Behaviour

The environmental fate of chemicals describes the processes by which they move and are transformed in the environment. In this section, some general characteristics of the Azo Solvent Dyes will be discussed with respect to their environmental fate in different compartments in an effort to understand how organisms come into contact with the substances in a particular medium. This discussion will include the persistence of these substances in environmental compartments, their degradation, their distribution among media, their removal from effluents by standard wastewater treatment methods and their bioaccumulation in organisms.

5.1 Environmental Distribution

As explained in Environment Canada and Health Canada (2013a), the Equilibrium Criterion model (EQC 2003) is not applicable to Azo Solvent Dyes, as they do not fall under the model domain. Therefore, the environmental fate and compartmentalization of these substances will be discussed qualitatively using information on their physical and chemical properties.

5.1.1 Water and sediment

If continuously released into water, some of the monoazo solvent dyes in the eco-subsets A and C may remain in the water column, given their water solubilities slightly above 1 mg/L. Others may remain in the water column up to their water solubility limits. Volatilization from water surfaces, especially turbulent shallow waters, may be an important fate process for azobenzene, given this substance's moderate vapour pressure (0.048 Pa) and moderate Henry's Law constant (1.37 $Pa \cdot m^3/mol$). However, volatilization from water surfaces is expected to be attenuated by adsorption to suspended solids and sediment in the water column (HSDB 1983–). In fact, the majority of Azo Solvent Dyes may have some affinity for the organic components of suspended solids. Instead of electrostatic interactions, the non-ionic forms of these hydrophobic dyes may associate with or bind to organic matter in the water column due to hydrophobic interactions and eventually may settle out to sediments or wastewater sludge (particularly as a substance's molecular weight increases). This is consistent with a study that demonstrated that disperse dyes can bind to small particle size fractions of certain treated adsorbent minerals (e.g., calcined alunite; Özacar and Şengil 2002). In considering that the higher molecular weight Azo Solvent Dyes may be acting as particles, and given their low water solubilities, they are ultimately expected to fall out of the water column and settle in sediment.

5.1.2 Soil

There are two major routes for the release of azo dyes to soil: directly via the use or application of a dye in the environment and indirectly via the application of wastewater biosolids to agricultural land or deposition in landfills. If released to soil, these Azo

Solvent Dyes are expected to have low mobility in the soil, as they will tend to adsorb to soil particles, given their hydrophobic nature. Modelled organic carbon–water partition coefficients for the monoazo solvent dyes were moderate to high (log K_{oc} values of > 2.5; Appendix B, Table B-4), indicating that these substances would not be very mobile in soils (or sediment) due to their affiliation with the organic carbon component. Experimental K_{oc} values for azobenzene ranged from 1350 to 4510 (HSDB 1983–), indicating that this substance may have low to slight mobility in soils. Given the size and complexity of the disazo solvent dyes, it is expected that they would be even less mobile than the monoazo solvent dyes.

Volatilization from moist soil surfaces may occur for azobenzene, based on this substance's water solubility (~6.4 mg/L), moderate vapour pressure (0.048 Pa) and moderate Henry's Law constant (1.37 $Pa \cdot m^3/mol$) (HSDB 1983–). However, this process may be attenuated by its affinity to soil organic matter. In general, this process is not expected to be an important fate process for the Azo Solvent Dyes.

These Azo Solvent Dyes would be associated with biosolids if released to local wastewater treatment systems. Although no data were available pertaining to the measurement of these substances in biosolids, effluents from a number of local wastewater treatment systems across Canada were sampled from 2009 to 2012 as part of the CMP Monitoring and Surveillance Program. Samples were analyzed for three Azo Solvent Dyes, including Solvent Red 1, Magneson II and Solvent Red 23, and none of these substances was detected (2012 email from Aquatic Ecosystem Research Protection Division, Water Science and Technology Directorate, Environment Canada, to Environmental Assessment Division, Science and Risk Assessment Directorate, Environment Canada; unreferenced). Various treatment processes were used (including primary, secondary and lagoon treatment), depending on the wastewater treatment plant sampled, and it was not confirmed whether industrial activities related to these solvent dyes were releasing effluents to any of these systems.

5.1.3 Air

The limited experimental data and modelled data available indicate that these Azo Solvent Dyes have low vapour pressures and low Henry's Law constants, with values decreasing as molecular weights increase. One of the monazo solvent dyes, azobenzene, is somewhat different in that it has a moderate vapour pressure (0.048 Pa) and a moderate Henry's Law constant (1.37 $Pa \cdot m^3/mol$). If released to air, it is expected to exist solely as a vapour, but is not expected to remain in the vapour phase due to abiotic degradation. Therefore, it is not expected to be transported far from the source. Considering the low water solubilities of these Azo Solvent Dyes and the molecular weights (> 300 g/mol) of many of these substances, it is expected that they would associate with particulate matter and be deposited near the source. Therefore, given their physical and chemical properties, the Azo Solvent Dyes would have a low potential for long-range atmospheric transport if they were to be released to air.

5.2 Environmental Persistence

In order to characterize the environmental persistence of the Azo Solvent Dyes, available empirical and modelled data on abiotic and biotic degradation were considered. Experimental and modelled biodegradation data for the Azo Solvent Dyes were considered for both aerobic and anaerobic conditions. Atmospheric oxidation is predicted to be an important fate process for these substances if they are released to the atmosphere. However, hydrolysis is not expected to be an important factor in the aquatic environment, as these substances do not contain any hydrolyzable groups. In addition, the process of ecological biotransformation is considered with respect to the potential for the Azo Solvent Dyes to degrade to aromatic amines as a result of cleavage of the azo bond under anaerobic or reducing conditions.

5.2.1 Empirical biodegradation data

Empirical biodegradation data related to the persistence of the Azo Solvent Dyes are limited. Data were available for only three monoazo substances in eco-subset A. Empirical tests were conducted under aerobic conditions. Some representative studies are summarized in Table 5-1.

Table 5-1. Empirical data for degradation of the Azo Solvent Dyes (some substances in eco-subset A) under aerobic conditions

CAS RN	Medium	Fate process	Degradation value	Degradation endpoint/units	Reference
Solvent Yellow 1	Wastewater	Biodegradation	89% (50 mg/L)	% biodegradation—13 days	Urushigawa and Yonezawa 1977
	Wastewater	Biodegradation	0% (BOD)	% biodegradation—5 and 6 days	Heukelekian and Rand 1955
	Water	Biodegradation	25.8% (low-nitrogen culture) 4.7% (high-nitrogen culture)	% biodegradation—12 days	Spadaro et al. 1992
	Wastewater	Biodegradation	46% (HPLC) (100 ppm)	% biodegradation—24 h	Idaka and Ogawa 1978
	Wastewater	Biodegradation	59% (HPLC) (100 ppm)	% biodegradation—48 h	Idaka and Ogawa 1978
	Water	Biodegradation	89%	% biodegradation—13 days	HSDB 1983–
Solvent Yellow 2	Water	Biodegradation	100%	% biodegradation—28 days	Fochtman 1981
	Water	Biodegradation	100%	% biodegradation—7 days	HSDB 1983–

CAS RN	Medium	Fate process	Degradation value	Degradation endpoint/units	Reference
Azo-benzene	Water	Biodegradation	50%	% biodegradation—1.4 days	Zoeteman et al. 1980
	Water	Biodegradation	50%	% biodegradation—13.8 h	Weber and Wolfe 1987
	Wastewater	Oxidation	225 mg/L	Oxygen uptake	Malaney 1960

Abbreviations: BOD, biological oxygen demand; CAS RN, Chemical Abstracts Service Registry Number; HPLC, high-performance liquid chromatography; ppm, parts per million

These data indicate some conflicting results for biodegradation of the tested substances. The interpretation of the results is complicated by the use of different media and lengths of tests. Nitrogen limitation also resulted in faster biodegradation of select azo-based solvent dyes (Spadaro et al. 1992). Furthermore, it is unclear whether or not commercial formulations were used instead of high-purity dyes. Although many of these results indicate that these monoazo substances are readily biodegraded, tests that determine ultimate degradation (i.e., mineralization) are also needed to demonstrate whether these substances would persist in the environment. Additional studies on these three monoazo solvent dyes can be found in Appendix C, Table C-1.

Studies on the persistence of Azo Solvent Dyes under anaerobic conditions are lacking. The half-lives of Solvent Red 1 were 2.2 days and 4 days in sediments from two different lakes. Measured breakdown products from the cleavage of the azo bond were *o*-anisidine (CAS RN 90-04-0) and l-amino-2-naphthol (CAS RN 2834-92-6) (Baughman and Weber 1994). If Azo Solvent Dyes accumulate in anoxic sediments, they may undergo biodegradation (Yen et al. 1991) to potentially harmful aromatic amines, depending on how tightly these substances are bound to the solids and organic matter. Some substances may bind reversibly and become resuspended, while others may bind irreversibly and become buried in the anoxic layers. Most organisms would not be exposed to potentially harmful transformation products in deep anoxic sediments where these amines would be formed. The amine degradation products may be tightly bound to sediments, further limiting their bioavailability (Weber et al. 2001; Colon et al. 2002).

Experimental evidence from Chen et al. (2009) showed that two bacteria were able to transform Solvent Red 23 and Solvent Red 24 to aniline (CAS RN 62-53-3) and *o*-toluidine (CAS RN 95-53-4), respectively, under anaerobic conditions. It is noted that the bacteria were acclimated to both chemicals, as they were incubated under anaerobic conditions in the presence of 1.5 mg/mL of each dye at 37°C for 36 hours (Chen et al. 2009). In another study, the chemical reduction of Solvent Red 23 under alkaline conditions demonstrated that both aniline and *o*-toluidine could be released as breakdown products (Pielesz et al. 2002).

The persistence of Azo Solvent Dyes in sediments would also depend on whether the release of these substances to water is continuous, the rate at which these substances settled to sediments, and the mixing and resuspension of sediments due to water

patterns. The significance of the biodegradation of azo compounds in sediments is further discussed in Environment Canada and Health Canada (2013a).

5.2.2 Modelling of persistence

Because of the limited empirical data, (Q)SAR models were also used to evaluate the persistence of the Azo Solvent Dyes. These models examine the inherent ability of a substance to be degraded to base minerals by microorganisms under standard laboratory testing conditions, based on the susceptibility to degradation of its structural fragments. The use of the models assumes complete bioavailability and accessibility of the Azo Solvent Dye as a single molecule in aqueous sludge slurry. There is some uncertainty with these estimates, given that full bioavailability and accessibility may not be realized under many environmental conditions and that some of the less soluble dyes may exist as solid particles. Therefore, the (Q)SAR predictions represent optimal environmental conditions for biodegradation.

Appendix C (Tables C-2 and C-3) and Environment Canada (2013a) summarize the results of available (Q)SAR models for degradation of the individual Azo Solvent Dyes in various environmental media. Aquatic degradation models used in these analyses were HYDROWIN (2010), BIOWIN Sub-models 3–6 (BIOWIN 2010), DS TOPKAT (©2005–2009) and CATALOGIC (2012). Most of the model outputs for the Azo Solvent Dyes predicted that they would biodegrade slowly (eco-subsets A, B, D, E, F, G and H) or that they were near the cut-off for degrading slowly versus moderate degradation (eco-subset C) in sewage water under aerobic conditions. These results are consistent with information discussed elsewhere (Environment Canada and Health Canada 2013a) that outlines the general persistence of azo dyes in aerobic environments. All substances but one (azobenzene) were found to have the potential to degrade quickly in air, should releases occur to that medium. Although there is some uncertainty in the modelled values for the disazo solvent dyes due to the assumption that they are behaving as single molecules, the extrapolated half-lives and overall conclusion are logical given that these substances would be expected to be even more persistent if they were behaving as solid particles.

5.2.3 Summary of persistence

Based on empirical and modelled data, the Azo Solvent Dyes assessed in this report do not persist in air. According to modelled data, azobenzene may tend to persist in air; however, experimental data suggest that it would not persist more than a couple of days. Fifteen of the 22 Azo Solvent Dyes (eco-subsets A, B, D, E, F, G and H) tend to persist in water, sediment and soil. The seven monoazo solvent dyes in eco-subset C also tend to persist in water, sediment and soil, but less so than those in the other eco-subsets.

5.3 Potential for Bioaccumulation

In this assessment, a variety of lines of evidence have been used to determine the bioaccumulation potential of the Azo Solvent Dyes. Experimental data for traditional bioaccumulation metrics such as bioconcentration factors (BCFs) or bioaccumulation factors (BAFs) are minimal and restricted to the water compartment for these substances. The use of bioaccumulation modelling was incorporated as an additional line of evidence.

5.3.1 Octanol–water partition coefficient

As indicated in Table 3-1 and Table 3-2, the Azo Solvent Dyes have a range of experimental water solubilities, from practically insoluble to sparingly soluble (3.3×10^{-4} – 34 mg/L). Experimental log K_{ow} values for the monoazo solvent dyes in eco-subset A (1.5–4.6) and the experimental log K_{ow} of 7.5 for one of the disazo solvent dyes are moderate to high, suggesting some potential for diffusion into organisms according to equilibrium partitioning theory.

5.3.2 Bioconcentration factors (BCFs)

The limited bioconcentration data available for the Azo Solvent Dyes (Table 5-2) illustrate low BCF values. In addition to the limited bioconcentration data, BCF data available for three analogues of the disazo solvent dyes (Table 5-3) were also considered in the weight of evidence.

Table 5-2. Empirical bioconcentration data for the Azo Solvent Dyes

CAS RN, C.I. name (eco-subset)	Test organism	Experimental concentration (duration)	Endpoint (BCF, L/kg)	Reference
Solvent Yellow 1 (A)	Common carp (*Cyprinus carpio*)	6 µg/L (28 days)	37.3	CHRIP ©2008
	Common carp (*Cyprinus carpio*)	0.6 µg/L (28 days)	< 31.6	CHRIP ©2008
Solvent Red 24 (G)	Common carp (*Cyprinus carpio*)	0.35 mg/L (42 days)	< 0.29–2.9	MITI 1992
	Common carp (*Cyprinus carpio*)	0.035 mg/L (42 days)	< 2.9–11	MITI 1992

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

Table 5-3. Empirical bioconcentration data for analogues of the disazo solvent dyes

CAS RN, C.I. name (eco-subset)	Test organism	Experimental concentration (duration)	Endpoint (BCF, L/kg)	Reference
Disperse Orange 73 (E)	Common carp (*Cyprinus carpio*) (4.3% lipid)	0.1 mg/L (42 days)	0.8–14	MITI 1992
Disperse Orange 73 (E)	Common carp (*Cyprinus carpio*) (4.3% lipid)	0.01 mg/L (42 days)	< 2.5–11	MITI 1992
Disperse Red 167 (G)	Common carp (*Cyprinus carpio*) (4.3% lipid)	0.1 mg/L (42 days)	< 0.3–1.1	MITI 1992
Disperse Red 167 (G)	Common carp (*Cyprinus carpio*) (4.3% lipid)	0.01 mg/L (42 days)	< 3.3–7.4	MITI 1992
Disperse Yellow 163 (G)	Common carp (*Cyprinus carpio*) (3.9% lipid)	0.1 mg/L (42 days)	30–49	MITI 1992
Disperse Yellow 163 (G)	Common carp (*Cyprinus carpio*) (3.9% lipid)	0.01 mg/L (42 days)	26–47	MITI 1992

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

In addition, the use of bioaccumulation models was deemed acceptable for the monoazo solvent dyes based on their relatively simple chemical structures. The output and results can be found in Environment Canada (2013a). Modelled data show limited potential for bioaccumulation for most of the substances, with substances in eco-subset C (particularly Solvent Red 1, Solvent Orange 2, Solvent Red 4 and Solvent Orange 7) showing more potential to bioaccumulate than the other monoazo solvent dyes. Eco-subsets E and F also showed a higher potential to bioaccumulate; however, due to the more complex chemical structures of these substances and the uncertainty as to whether or not they exist as single molecules, less confidence is placed in the modelling results for the disazo solvent dyes. However, assuming that these substances are behaving as single molecules, the modelled data may help to determine a worst-case scenario for potential bioaccumulation. The data also indicate that metabolism is a significant factor in reducing the potential for bioaccumulation.

5.3.3 Other factors for assessing bioaccumulation potential

In terms of bioaccumulation, it is also useful to consider molecular size and cross-sectional diameter, which are parameters commonly used by international jurisdictions in weight of evidence conclusions on bioaccumulation potential. For example, ECHA (2008) shows that some additional indicators for low bioaccumulation potential might be applicable for substances with low solubility in *n*-octanol and water. In particular, a maximum cross-sectional diameter (D_{max} average) greater than 1.7 nm may be considered as an additional indicator.

Investigations relating fish BCF data to molecular size parameters (Dimitrov et al. 2002, 2005) suggest that the probability of a molecule crossing cell membranes as a result of passive diffusion declines significantly with increasing maximum diameter (D_{max}). The probability of passive diffusion decreases appreciably when the maximum diameter is greater than about 1.5 nm, and even more so for molecules having a maximum diameter of greater than 1.7 nm. Sakuratani et al. (2008) also investigated the effect of cross-sectional diameter on passive diffusion in a BCF test set of about 1200 new and existing chemicals. They observed that substances that do not have a very high bioconcentration potential (i.e., BCF < 5000) often have a D_{max} of greater than 2.0 nm and an effective diameter (D_{eff}) of greater than 1.1 nm. Anliker et al. (1988) also proposed that a second largest cross-sectional diameter of more than 1.05 nm with a molecular weight of greater than 450 g/mol would suggest a lack of bioconcentration for organic colourants. Therefore, D_{eff} values of greater than 1.05–1.1 nm and D_{max} values of greater than 1.5–1.7 nm can be considered indicators of a reduced rate of uptake from water. A reduced rate of uptake allows other internal elimination processes, such as metabolism and fecal egestion, to reduce the overall chemical burden in the tissues of organisms, reducing bioaccumulation on a whole-body basis.

Due to the lack of empirical bioaccumulation data available for the Azo Solvent Dyes, available data on water solubility, molecular weight and cross-sectional diameter are considered in order to determine the bioaccumulation potential of these substances.

The monoazo solvent dyes were found to have effective cross-sectional diameters that ranged from 0.65 nm ($D_{eff\text{-}min}$) to 1.1 nm ($D_{eff\text{-}max}$), with average effective cross-sectional diameters ranging from 0.69 to 0.97 nm (Environment Canada 2014). The disazo solvent dyes had effective cross-sectional diameters that ranged from 0.86 nm ($D_{eff\text{-}min}$) to 1.56 nm ($D_{eff\text{-}max}$), with average effective cross-sectional diameters ranging from 0.95 to 1.33 nm (Environment Canada 2014). The range in maximum cross-sectional diameters ($D_{min\text{-}max}$) was 1.06 to 1.81 nm for the monoazo solvent dyes and 1.26 to 2.99 nm for the disazo solvent dyes (Environment Canada 2014). The monoazo solvent dyes have low molecular weights (182–306 g/mol), while the molecular weights for the disazo solvent dyes are higher (352–646 g/mol). In general, as the effective and maximum cross-sectional diameters increase, the rate of uptake of the substance across cell membranes is likely to decrease.

It should, however, be noted that according to Arnot et al. (2010), there are some uncertainties associated with the thresholds proposed by Dimitrov et al. (2002, 2005) and Sakuratani et al. (2008), as the bioaccumulation studies used to derive them were not always critically evaluated. Arnot et al. (2010) pointed out that molecular size influences solubility and diffusivity in water and organic phases (membranes), and larger molecules may have slower uptake rates. However, these same kinetic constraints apply to diffusive routes of chemical elimination (i.e., slow uptake = slow elimination). Thus, significant bioaccumulation potential may remain for substances that are subject to slow absorption processes, if they are slowly biotransformed or slowly eliminated by other processes. However, if the rate of gill uptake is sufficiently mitigated

by steric hindrance to the point that the rate of elimination exceeds uptake, bioconcentration will be lowered.

5.3.4 Summary of bioaccumulation potential

The Azo Solvent Dyes are expected to have a low bioaccumulation potential due to low observed bioconcentration in empirical tests. Due to the paucity of experimental data across the different eco-subsets, additional data from disperse dyes were used (when applicable). The low bioaccumulation potential of Azo Solvent Dyes was supported by modelled results, especially when metabolism was considered. There is uncertainty in these results for eco-subset C, as there were no experimental data available for these substances, and the modelling results indicate that these substances may have the potential to bioaccumulate. This indication is supported by the physical and chemical properties of these substances (i.e., lower molecular weights, smaller cross-sectional diameters, one substance with an experimental log K_{ow} of 7.5).

Data were also unavailable regarding the bioaccumulation of Azo Solvent Dyes from exposures to these substances in soil or sediment.

6. Potential to Cause Ecological Harm

6.1 Ecological Effects Assessment

Ecological effects of the Azo Solvent Dyes were characterized based on the empirical data available for these substances. Empirical data for analogues have also been taken into consideration in this assessment. A critical body burden (CBB) approach was also considered as part of the weight of evidence.

6.1.1 Ecotoxicity in the aquatic compartment

Ecotoxicity data in the aquatic compartment for the Azo Solvent Dyes and their analogues are summarized in Appendix D (Tables D-1 and D-2). Substance identity information for the analogues chosen, along with physical and chemical property information (where available), is summarized in Appendices A and B. Empirical data were available only for the monoazo solvent dyes in eco-subsets A (Solvent Yellow 1, Solvent Yellow 2, azobenzene and Solvent Orange 3) and C (preliminary studies on Solvent Red 1). In addition, empirical data were available for eight analogues, including Solvent Yellow 5, Solvent Yellow 6 and Solvent Yellow 7 (eco-subset A); and Direct Red 81 (eco-subset H). Information on chronic studies is limited.

It is noted that some Azo Solvent Dyes are somewhat poorly soluble in water and can be difficult to test in this medium, as they may not dissolve naturally. Mechanical (i.e., using a saturator system) or chemical means (i.e., using carriers, solvents or dispersing agents) may be used in studies to facilitate dissolution and support stable dispersions. As a result, some of the test chemical concentrations tend to be above their water solubilities. Such concentrations of Azo Solvent Dyes are not likely realistic in the Canadian environment, even with surfactants contained in commercial dye products. Surfactants, with other factors (i.e., temperature and pressure), may affect the solubility of the chemicals in the environment; however, these factors are not expected to enhance water solubility more than 3 orders of magnitude over solubility under laboratory conditions. Organisation for Economic Co-operation and Development (OECD) test guidelines state that the use of any auxiliary agent at a low concentration should not cause additional toxic effects on the test organisms. If toxic effects are apparent, these should be identified and eliminated from the study by use of a solvent control group.

However, it has also been pointed out (Rufli et al. 1998) that these auxiliary agents, even if not toxic, may have a pronounced effect on the physical form of the hydrophobic substances by influencing their bioavailability in the test medium. Therefore, results from an ecotoxicological experiment using any auxiliary agent may be applicable only to a defined substance–dispersant system, making it difficult to extrapolate to other exposure conditions. The solvent controls containing dispersant can be used to identify only dispersant-related effects, not dispersant–substance interactions (Rufli et al. 1998).

In addition, test concentrations far above the water solubility of the test substance may also result in more soluble impurities that may influence the toxicity results, thus confusing the interpretation of the toxicity of the substance of concern (Weyman et al. 2012). Weyman et al. (2012) indicated that in toxicity texts where a solvent is used, the undissolved test material has the potential to exert adverse (physical) effects on test organisms, such as the blocking of fish gill membranes, encapsulation/entrapment of daphnids or the reduction of light intensity in algal tests. All of these factors confound the results of the toxicity tests and need to be taken into consideration when interpreting the data for use in the ecological assessment.

Ecotoxicological experiments on Azo Solvent Dyes have been conducted using a variety of aquatic organisms, with a variety of toxicological responses observed, depending on the organisms and the particular substances that were studied (see Appendix D, Tables D-1 and D-2). Available empirical toxicity information for substances in eco-subsets A and C indicate that these substances are highly (median lethal concentration [LC_{50}] values < 1 mg/L) to moderately (LC_{50}s 1–10 mg/L) toxic to aquatic organisms. Key studies considered in choosing a critical toxicity value are summarized in Table 6-1 and Table 6-2.

Table 6-1. Empirical toxicity data for the Azo Solvent Dyes: key studies considered in choosing a critical toxicity value

Chemical	Species name (organism)	Test type	Endpoint (mg/L)	Reference
Solvent Yellow 1	*Oryzias latipes* (fish)	96 h LC_{50}	0.23	CHRIP ©2008
	Pseudokirchneriella subcapitata (alga)	48 h EC_{50}[a]	0.46	CHRIP ©2008
Azobenzene	*Scenedesmus subspicatus* (alga)	0–48 h EC_{50}[b]	1.7	Kuhn and Pattard 1990
Solvent Orange 3	*Oryzias latipes* (fish)	48 h LC_{50}	0.3	Tonogai et al. 1982
Solvent Red 1	*Hyalella azteca* (invertebrate)	1-week LC_{50}	0.277[c]	Bartlett 2014
	Hyalella azteca (invertebrate)	4-week LC_{50}	0.0265[c]	Bartlett 2014
	Pimephales promelas (fish larvae)	20-day LC_{50}	0.0167	Parrott et al. 2014

Abbreviations: EC_{50}, the concentration of a substance at which there is a sublethal effect observed on 50% of the test organisms within the test duration; LC_{50}, the concentration of a substance at which there is a lethal effect observed on 50% of the test organisms within the test duration

[a] Immobilization.

[b] Reduction in cell growth.

[c] Nominal concentration from preliminary internal report; lowest LC_{50} concentration from multiple 1-week and 4-week experiments.

Table 6-2. Empirical toxicity data for analogues of the Azo Solvent Dyes: key studies considered in choosing a critical toxicity value

Chemical	Organism	Test type	Endpoint (mg/L)	Reference
Solvent Yellow 6	*Oryzias latipes* (fish)	48 h LC_{50}	0.4	Tonogai et al. 1982
Solvent Yellow 7	*Pimephales promelas* (fish)	72–96 h LC_{50}	1.10	Holcombe et al. 1984

Chemical	Organism	Test type	Endpoint (mg/L)	Reference
Solvent Yellow 7	*Pimephales promelas* (fish)	96 h LC_{50}	1.17	Russom et al. 1997

Abbreviation: LC_{50}, the concentration of a substance at which there is a lethal effect observed on 50% of the test organisms within the test duration

In general, aquatic invertebrates may be slightly more sensitive to Azo Solvent Dyes than other organisms. Results of acute and chronic aquatic toxicity studies on fish, invertebrates and algae were all within a range from approximately 0.02 to 6.38 mg/L (data for analogues also fell within this range).

Chronic data for fish, invertebrates and algae are limited. Acute and chronic exposures of *Hyallela azteca* to Sudan Red G (or Solvent Red 1) were conducted using a carrier (methanol) to improve the solubility of the substance in water (Bartlett 2014). Preliminary test results are based on nominal concentrations and multiple survival and growth experiments were conducted over the course of 4 weeks. Comparison of acute and chronic endpoints (i.e., LC_{50}) of the study indicates that chronic exposures result in a 10-fold decrease in the effect concentration. In another chronic study (Parrott et al. 2014), fathead minnow (*Pimephales promelas*) embryo-larval tests were conducted with Solvent Red 1. Daily static-renewal exposures began at 0 days post-hatch and ended at 14 days post-hatch, for a 20-day exposure period (with hatch at 4–5 days post-fertilization). Methanol was used as a carrier solvent, but organism survival was not affected in control treatments. It was noted during the test that it was difficult to keep the dye in solution, as it was observed at the top of the beaker and on test equipment. Larval fish were also observed to be eating red dye precipitate at the highest exposure concentrations. In fact, measured concentrations were observed to drop significantly compared with nominal concentrations after 24 hours in the beaker. A chronic LC_{50} of 0.0167 mg/L was reported for fathead minnow larval survival (Parrott et al. 2014).

Some information (Khudoley 1972 and other references cited in that paper) is available linking exposure of fish to Azo Solvent Dyes (including Solvent Yellow 2 and Solvent Yellow 3) through either food or water with changes in hepatocytes, pretumorous conditions in the kidney and behavioural changes early in the exposures. As mentioned previously, cleavage of the azo bond under anaerobic or reducing conditions (e.g., deep layers of sediments) is known to result in the production of aromatic amines, some of which are known or potential carcinogens. The metabolic degradation of azo substances in the gut can also result in the formation of potentially harmful aromatic amines. The bioavailability of such substances would control their ultimate toxicity in the organism. These results are consistent with the information presented in the Health Effects Assessment section regarding the carcinogenic and genotoxic potential of these substances.

Given the similarity in structures of substances within eco-subsets A, B, C and E, it is likely that these Azo Solvent Dyes all have a common mode of action based on the amine, aniline or phenolic functional groups resulting from biotransformation of the parent structures. The two substances in eco-subsets D and F (having the pyrazolone

ring) are also expected to behave similarly given their structures, although no empirical toxicity information was available for either of these substances. Substances in eco-subset G have much more complex structures than these other Azo Solvent Dyes. However, given the lack of any empirical data for these substances, they are conservatively presumed to have the same mode of action as the other substances. The mode of action for all of the substances in eco-subsets A through G, although not known for certain, could be polar narcosis or some other reactive mode of action, which means that these substances would be more toxic than baseline narcosis would predict. Tosato et al. (1993) found that azobenzene was more toxic to *Daphnia* than predicted. Schultz (1997) tested the analogue Solvent Yellow 7 on a freshwater ciliate and categorized it as having a bioreactive mode of action. Russom et al. (1997) listed Solvent Yellow 7 as an oxidative phosphorylation uncoupler with a high degree of confidence.

The UVCB (CAS RN 73507-36-5) in eco-subset H is not expected to behave like the other Azo Solvent Dyes in this subgroup, as it is a salt and will dissociate into its subcomponents in water. One of its main components appears to be similar to Direct Red 81. One empirical study for Direct Red 81 reported a 96 h TL_{50} (median threshold limit) for fathead minnows (*Pimephales promelas*) of > 180 mg/L (Little and Lamb 1972). Given this result, it would be expected that CAS RN 73507-36-5 would not be highly toxic to aquatic organisms.

6.1.1.1 Estimated Critical Body Burden (CBB)

According to their physical-chemical properties and results of bioaccumulation studies, most Azo Solvent Dyes are considered to have low solubilities in water and low potential to bioaccumulate in non-human organisms. Based on the empirical data identified for Azo Solvent Dyes, these substances are expected to have low toxicities to fish at saturation in water. However, there is still a question regarding the ecological effects of these substances on aquatic organisms related to their water solubility. This is because there may be different solubilities, bioavailabilities and inherent toxicities of Azo Solvent Dyes as pure substances compared with those forms containing impurities or other formulations.

To help answer this question, a critical body burden (CBB) or internal critical concentration (ICC) approach can be applied as a “check mechanism.” In this situation, the external acute effect concentrations of substances causing the mortality of organisms can be calculated and compared with the results of ecotoxicity studies with pronounced biological effects (i.e., tests with solvents/dispersants) and ecotoxicity classification schemes (i.e., LC_{50}/EC_{50} values reflecting low, moderate or high aquatic toxicity levels).

This approach is described in detail in Appendix E of this report, and calculated external acute effect concentrations for two Azo Solvent Dyes and three analogues (for which the lipid contents of test organisms have been reported) are summarized in Table 6-3.

Table 6-3. Calculated external acute effect concentrations (LC_{50}s) for Azo Solvent Dyes and disperse dye analogues using the CBB approach

C.I. Name (eco-subset)	Molecular weight (g/mol)	External acute effect concentration (LC_{50}, mg/L)
Solvent Yellow 1 (A)	197.24	28.6
Solvent Red 24 (E)	380.45	445.23
Disperse Red 167 (G)	520	739.44
Disperse Yellow 163 (G)	417	42.80
Disperse Orange 73 (E)	443	269.24

Calculated results indicate that to reach the CBB threshold levels and cause 50% mortality in the test organisms, exposure concentrations of Azo Solvent Dyes would be above 28.6 mg/L (LC_{50} ≥ 28.6 mg/L). This estimate suggests that these substances are moderately toxic to fish. This is in line with the low bioaccumulation potential in fish, as indicated in the Potential for Bioaccumulation section, where it was hypothesized that bioavailability is not expected to be high for substances in this subgroup. Indeed, very low BCFs (i.e., little uptake) of these sparingly soluble (in water) substances mean less chance for them to demonstrate toxic effects. This point is confirmed by the above-mentioned CBB-based external effect concentrations and by the lack of significant adverse effects observed in any of the bioconcentration tests.

6.1.1.2 Determination of assessment factor and Predicted No-Effect Concentration (PNEC) in the aquatic compartment

Given the likelihood that the substances in eco-subsets A through G all have a common mode of action and given that they are expected to cause harm to aquatic organisms at low concentrations, a predicted no-effect concentration (PNEC) is derived for all of these substances. In order to determine a PNEC, the most sensitive (reliable) endpoint was chosen as a critical toxicity value, in considering the acceptability of available studies. This critical toxicity value is divided by an assessment factor, which is chosen to account for acute versus chronic endpoints and interspecies variability.

The critical toxicity value chosen is 0.23 mg/L, based on a 96-hour LC_{50} for the fish species *Oryzias latipes* exposed to Solvent Yellow 1 (CHRIP ©2008). This value was chosen because it was one of the most sensitive studies among those available for the Azo Solvent Dyes. It was considered to be a reliable study, it used standard methodology and the toxicity value was within the water solubility range for the substance. Two of the acute studies (BUA 2000; Tosato et al. 1993) with values slightly lower than this one, along with the chronic study by Parrott et al. (2014), were deemed to be of slightly lower reliability, but were still considered in the weight of evidence. Given the multiple toxicity values available for different species and the fact that this toxicity value is within the water solubility range of the substance, an assessment factor of 100 is applied to this critical toxicity value to derive the PNEC.

Therefore, the PNEC for water = 0.23 mg/L / 100 = 0.0023 mg/L or 2.3 µg/L

6.1.2 Empirical studies for the soil compartment

There is little information from toxicological studies of Azo Solvent Dyes in soil. Milani (2013) conducted a range-finding study and reported ecotoxicity data for Sudan Red G (or Solvent Red 1) on turtle embryos (*Chelydra serpentina*). Within a series of six concentration groups, a 100% hatching success rate was observed in three test groups: 0, 5 and 124 mg/kg soil (dry weight). In the test groups of 1, 25 and 625 mg/kg soil (dry weight), the hatching success rate was reported as 92.5%, 90% and 97.5%, respectively. The study is continuing to collect data to determine the potential for effects on the development of these embryos (Milani 2013). Although this information is preliminary, it suggests that Azo Solvent Dyes may not be harmful to soil-dwelling organisms, likely as a result of their low bioavailability in this medium.

Since the above data are preliminary, they are not considered suitable for use as a critical toxicity value. Therefore, a PNEC has not been calculated for the soil compartment.

6.1.3 Empirical studies for the sediment compartment

No published ecotoxicity data on sediment organisms have been identified for Azo Solvent Dyes or their analogues. Milani et al. (2014) conducted studies on the effects of Sudan Red G (or Solvent Red 1) on mayflies (*Hexagenia* spp.) in a 21-day test and on an oligochaete worm (*Tubifex tubifex*) in a 28-day test in which organisms were exposed to concentrations of Solvent Red 1 in spiked sediments. In range-finding tests for mayflies, no effects on survival were observed up to average Solvent Red 1 concentrations of 191 µg/g dry weight (associated nominal concentration was 1000 µg/g, the highest concentration tested). A slight reduction in growth was noted at this concentration. *Tubifex tubifex* was more sensitive in a definitive test, with an LC_{50} of 6.88 µg/g (dry weight). *Tubifex tubifex* reproduction endpoints were more sensitive than survival, with IC_{25}s of 1.24 µg/g for cocoon production and 0.82 µg/g for young production. No young were present at average measured concentrations of 16.01 µg/g. Measured concentrations of Solvent Red 1 in sediment were 35 to 48% of nominal concentrations in the tests. This preliminary information suggests that Azo Solvent Dyes may be hazardous to sensitive sediment-dwelling organisms at low concentrations in sediment.

Since the above data are preliminary, they are not considered suitable for use as a critical toxicity value. Therefore, a PNEC has not been calculated for the sediment compartment.

6.1.4 Ecological effects summary

Based on lines of evidence involving empirical and read-across aquatic ecotoxicity data, it may be expected that the Azo Solvent Dyes (with the exception of CAS RN 73507-36-5) can cause harm to aquatic organisms at low concentrations, given sufficient bioavailability. Data for soil- and sediment-dwelling organisms are limited. The

preliminary soil and sediment toxicity data indicate that the Azo Solvent Dyes are unlikely to be harmful to soil-dwelling organisms, but may be hazardous to sensitive sediment-dwelling organisms at low concentrations in sediment. There is also the potential for the breakdown products of some of these substances to be genotoxic or carcinogenic to aquatic organisms.

6.2 Ecological Exposure Assessment

6.2.1 Measured environmental concentrations

Because of difficulties in the determination of different classes of dyes at trace levels in environmental samples, little is known of the environmental occurrence, persistence and fate of dyes (Maguire and Tkacz 1991). With respect to environmental monitoring data from other countries, results are also limited. No data are available on the environmental occurrence of dyes in Denmark (Øllgaard et al. 1998), and only a few studies have been conducted in the United States. In a study conducted by Shackleford and Cline (1983) and sponsored by the US Environmental Protection Agency (EPA), wastewater from 4000 industrial and publicly owned treatment works (or wastewater treatment systems) in the United States was analyzed for contaminants. This study detected Solvent Yellow 1 at a concentration of 522.7 µg/L in treated effluent from the organics and plastics industry. Other such studies have detected azobenzene at concentrations as high as 123 µg/L in effluents from automotive cleaning operations and other (unspecified) industrial laundering processes (Bursey and Pellizzari 1982). In contrast, an azobenzene concentration of only 0.03 µg/L was detected in effluent from a specialty chemical manufacturing plant (Jungclaus et al. 1978). During the US Geological Survey's National Water Quality Assessment, 536 streambed sites in 20 major river basin systems were sampled for environmental contaminants (Lopes and Furlong 2001). In this study, azobenzene and other semi-volatile compounds were detected at levels at or below 0.13 mg/kg (dry weight) sediment. Similar results were obtained in a study conducted on sediment from the Huaihe River in China, where azobenzene was detected in sediment at levels in the range of 0.13–0.29 mg/kg (Huang et al. 2004).

6.2.2 Releases to the environment

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

Anthropogenic releases of a substance to the environment depend upon various losses that occur during the manufacture, industrial, consumer or commercial[5] use and

[5] 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.

disposal of a substance. In order to estimate releases to the environment occurring at different stages of the life cycle of the solvent dyes, Environment Canada compiled information on the relevant sectors and product lines as well as emission factors[6] 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.

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).

6.2.3 Identification of important exposure scenarios

Quantitative exposure characterization is focused on exposure scenarios representing major environmental releases and relatively high levels of exposure. In general, the magnitude of release is a direct function of the quantity of a substance manufactured or used and its applicable emission factors. In cases where industrial releases are similar in quantity to consumer and/or commercial releases, the former normally results in higher levels of environmental exposure than the latter. This is because industrial releases are concentrated at a limited number of sites, while consumer and/or commercial releases are dispersed across the country.

Eight companies were identified as importing one or more of the Azo Solvent Dyes from a survey issued pursuant to section 71 of CEPA 1999 (reporting year 2005; Canada 2006) and the DSL Inventory Update survey (reporting year 2008; Canada 2009). Two companies reported their respective North American Industry Classification System (NAICS) codes as 812115 (beauty salons) and 44612 (cosmetics, beauty supplies and perfume stores). It was confirmed by the two companies that the Azo Solvent Dyes they imported were present in consumer/commercial products and were not used in industrial operations (2013 emails from companies to Environment Canada;

[6] An emission factor is generally expressed as the fraction of a substance released to a given medium, such as wastewater, land or air, during a life cycle stage, such as manufacture, processing, industrial application or commercial/consumer use. Sources of emission factors include emission scenario documents developed under the auspices of the OECD, data reported to Environment Canada's National Pollutant Release Inventory, industry-generated data, monitoring data, etc.

unreferenced). The two companies were therefore excluded from quantitative exposure analyses because they were not industrial users.

The types of activities for the other six companies were not clear from the data submissions (Canada 2006, 2009). The companies were therefore contacted by Environment Canada or Health Canada and requested to provide information needed for the exposure assessment. In response to this request, three companies indicated that the Azo Solvent Dyes they imported were already in consumer/commercial products (2013 emails from companies to Environment Canada and Health Canada; unreferenced). These three companies were therefore excluded from quantitative exposure analyses, as the solvent dyes they imported were not used in industrial operations.

In response to the same request, companies indicated that they distributed the imported Azo Solvent Dyes to downstream industrial users for incorporation into lubrication oil (2013 emails from companies to Environment Canada; unreferenced). In 2010, Environment Canada and Health Canada conducted a site visit to a lubrication oil production facility (Environment Canada and Health Canada 2010) and found that there were negligible environmental releases from the lubrication oil production. In particular, water was not used in the production or cleaning operations, and no wastewater was generated. Based on these findings, negligible environmental releases were expected from the production of lubrication oil in general, and the industrial users identified as lubrication oil producers were therefore excluded from quantitative exposure analysis.

Also in response to the same request, companies indicated that they distributed the imported Azo Solvent Dyes to downstream industrial users for incorporation into three types of products: 1) fuel oils and engine fuels, 2) adhesives and 3) lawn and garden care products (2013 emails from companies to Environment Canada; unreferenced). The information provided by a company through Environment Canada's New Substances Program indicated that closed and dedicated equipment was used to blend fuel additives into engine fuels, and environmental releases from this operation were not anticipated (Environment Canada 2009). Based on this information, the environmental releases from the blending of the imported solvent dyes as an additive into fuel oils were expected to be negligible and were therefore excluded from quantitative analysis. However, there were no available data indicating negligible releases from the production of adhesives or lawn and garden care products. Thus, estimates for the releases of and exposure to the imported solvent dyes from the production of these two types of products were provided.

Furthermore, in response to the same question, one company indicated that it formulated at its site the imported Azo Solvent Dyes into chemicals for use by textile mills (2013 email from company to Environment Canada; unreferenced). For the downstream industrial users (textile mills) identified by the company, the Azo Solvent Dyes imported were used as azo disperse dyes. Given the ultimate use as disperse dyes, this exposure analysis will be considered in the assessment of the Azo Disperse Dyes being conducted under the Substance Groupings Initiative.

To sum up, the following two scenarios were anticipated to generate potential environmental releases and were therefore selected for quantitative analysis:

1) formulation of adhesives;
2) formulation of lawn and garden care products.

6.2.4 Estimates for Predicted Environmental Concentrations (PECs)

Exposure to the Azo Solvent Dyes was estimated in the form of PECs. These concentrations were based on available information on the quantities of the Azo Solvent Dyes used, sector-specific emission factors, characteristics of the wastewater treatment systems involved and characteristics of the receiving environment. These PECs were estimated for water, sediment and soil. They are summarized below, and detailed calculations can be found in Appendix F.

6.2.4.1 Aquatic PECs

The aquatic PECs were estimated for the two scenarios identified above. The aquatic PEC was estimated based on a number of parameters, including the annual quantity of the Azo Solvent Dyes used, the number of annual operation days relevant to these solvent dyes, emission factor to wastewater, removal by wastewater treatment systems, wastewater flow and receiving water dilution. The approach used in the calculations was to determine the concentration of the Azo Solvent Dyes near the wastewater treatment system's effluent discharge point based on wastewater flow and receiving water dilution.

The aquatic PECs derived are based on the use quantities of the Azo Solvent Dyes, which were reported by the companies to be between 10 and 1000 kg/year (2013 emails from companies to Environment Canada; unreferenced). An emission factor to wastewater of 2% (default value) was used, as the actual emission factor was unknown. This default value is taken from guidance from the European Commission Joint Research Centre (ECJRC 2003). In addition, it was conservatively assumed that there was no on-site wastewater treatment, as there was no site-specific information available. Therefore, the aquatic PECs derived (0.40 µg/L and 0.46 µg/L) were considered to be conservative estimates.

6.2.4.2 Sediment PECs

An equilibrium sediment–water partition approach described by the European Chemicals Agency (ECHA 2010) was used to estimate the PECs of the Azo Solvent Dyes in sediment. This approach assumes that the concentration in bottom sediment is in equilibrium with the concentration in the overlying water. According to Gobas (2007), the concentration in the overlying water should pertain to the concentration in the aqueous phase and should not include quantities adsorbed to suspended sediment. The concentration in the aqueous phase can be estimated from the aquatic PEC, which is the total concentration in water. Thus, the sediment PECs for the two scenarios were

derived from their respective aquatic PECs and standardized to an organic carbon (OC) content of 3% for bottom sediment.

The sediment PECs (1.66 mg/kg and 1.94 mg/kg) are conservative because the concentrations in the aqueous phase were conservatively estimated. In the water column, the total concentration of the Azo Solvent Dyes or the aquatic PEC at a given site was a fixed value. It was split between the aqueous phase and the solids phase (suspended sediment). The concentration in the solids phase depended upon the OC content of the solids phase, and the latter varied from a low of 0.1 kg/kg to a high of 0.2 kg/kg (Gobas 2010). By selecting the low value of 0.1 kg/kg, the minimum concentration in the solids phase resulted, therefore yielding the maximum concentration in the aqueous phase. This maximum concentration then resulted in the maximum sediment PEC at a given site.

6.2.4.3 Soil PECs

An approach described by the European Chemicals Agency (ECHA 2010) was used to estimate soil PECs for the Azo Solvent Dyes resulting from the land application of sewage biosolids. Using this approach, the amounts of biosolids accumulated within the top 20 cm layer of soil over 10 consecutive years were estimated and used as the basis for soil PECs. One underlying assumption of the approach was that there was no loss due to degradation, volatilization, leaching or soil runoff. In general, this assumption resulted in conservative soil PECs.

The soil PECs estimated for the two scenarios (0.31 mg/kg and 0.59 mg/kg) using the above approach were each based on the amount of the solvent dyes sorbed to wastewater treatment sludge at a given wastewater treatment system for a given site. The amount sorbed to wastewater treatment sludge was conservatively estimated using the maximum sorption to wastewater treatment sludge determined by a computer model (ASTreat 2006). The use of the maximum sorption provided additional conservatism in the soil PECs derived.

6.3 Characterization of Ecological Risk

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

The Azo Solvent Dyes are generally hydrophobic substances that are sparingly soluble in water, with some monoazo substances in this subgroup having experimental water solubilities slightly above 1 mg/L. Given their import and use in Canada, potential releases to the aquatic environment and to the terrestrial environment (via wastewater treatment sludge) have been estimated. When considering potential releases to water,

sediment and soil, it is expected that the Azo Solvent Dyes may remain in the water column up to their apparent water solubility limit and may also partition to suspended solids/sediments or soil particles. Available experimental and modelled data regarding the abiotic and biotic degradation of the Azo Solvent Dyes indicate that these substances tend to persist in water, sediment and soil for months; however, some of the monoazo substances may degrade slightly faster than the other Azo Solvent Dyes. Under anaerobic or reducing conditions (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.

Although there are limited experimental data available, information (including modelled data) on the octanol–water partition coefficients (K_{ow} values) indicates a range from low log K_{ow} values (< 3.8) for a few of the monoazo solvent dyes to high log K_{ow} values (> 4.5) for the higher molecular weight substances, including the disazo solvent dyes (Environment Canada 2014). Fish BCFs were low for the two Azo Solvent Dyes and three analogues for which experimental data were available (representing both monoazo and disazo substances), indicating that these substances are not likely to bioconcentrate in aquatic organisms. These results were substantiated with modelled data that considered metabolism. However, modelled information for a few monoazo solvent dyes, including information on their physical and chemical properties (i.e., lower molecular weights, smaller cross-sectional diameters, log K_{ow} values), indicated that these substances may have the potential to bioaccumulate. Data were unavailable regarding the bioaccumulation of the Azo Solvent Dyes from exposures to these substances in soil or sediment.

The sole toxicity study available that relates to CAS RN 73507-36-5 (the UVCB) indicates that it would not be expected to be harmful to aquatic organisms at low concentrations. This substance was not reported to be in commerce above the reporting thresholds and thus is considered to represent a low risk.

All of the other Azo Solvent Dyes in eco-subsets A through G are expected to have a common mode of action, possibly polar narcosis or some other reactive mode of action (i.e., they are more toxic than baseline narcosis would predict), and data indicate that they are expected to be hazardous to aquatic organisms at low concentrations. Therefore, an aquatic PNEC was derived representing all of these substances. Sediment-dwelling organisms may also be adversely affected, although the available toxicological data are preliminary; thus, a sediment PNEC was not derived.

To assess the risks associated with these Azo Solvent Dyes (eco-subsets A through G), industrial exposure scenarios were developed based on the information received on the import and use of five of the Azo Solvent Dyes. The aquatic PECs were 0.40 and 0.46 µg/L. A risk quotient analysis was conducted by comparing these PECs to the aquatic PNEC of 2.3 µg/L. This analysis indicates risk quotients of about 0.2.

All of the other substances in eco-subsets A through G are not in commerce above the reporting thresholds, are in commerce above the reporting threshold but are imported in

a formulation product, or are in commerce above the reporting threshold but environmental releases were determined to be negligible. Therefore, it is expected that there is a low risk to the environment related to the Azo Solvent Dyes.

A risk quotient analysis was not conducted for the sediment and soil compartments, as there was insufficient information to develop a PNEC for these media. Preliminary toxicological data for Solvent Red 1 indicate that the Azo Solvent Dyes may not have the potential to cause harm to soil-dwelling organisms. Preliminary toxicological data for sediment indicates that Solvent Red 1 may have the potential to cause harm to sediment-dwelling organisms at low concentrations. Because the substances in eco-subsets A through G are expected to have a common mode of action, all of these substances may also have the potential to cause harm to sediment-dwelling organisms, based on the preliminary data.

Considering all available lines of evidence with respect to the persistence, potential for bioaccumulation, ecotoxicity, industrial uses and potential releases of the substances, it is concluded that the 21 Azo Solvent Dyes assessed in the ecological portion of this assessment have a low potential to cause ecological harm in Canada.

Due to the use of Solvent Yellow 77 (Disperse Yellow 3) in textile dye formulation and textile dyeing reported under section 71 of CEPA 1999, the ecological assessment and conclusion for this substance is summarized in the Azo Disperse Dyes assessment.

6.3.1 Uncertainties in evaluation of ecological risk

In general, with the exception of some of the monoazo solvent dyes, substances addressed in this report had limited data available. As a result, a read-across approach using data from selected analogues, including within and across eco-subsets, was the best alternative for estimating physical and chemical properties, persistence, the potential for bioaccumulation and toxicity.

This paucity of information necessitated the generation of model predictions for the physical and chemical properties of the monoazo solvent dyes and, in some cases, for the disazo solvent dyes as well. However, such information particularly that used for the disazo solvent dyes, was used with caution, given the uncertainty as to whether these substances are behaving as particles. No information was available on CAS RN 73507-36-5, with the exception of an aquatic toxicity study for Direct Red 81 (which is similar to one of the UVCB components). However, this substance was not reported to be in commerce above the reporting thresholds and thus is considered to represent a low risk.

Model predictions were required to fill data gaps for biodegradation, including for the disazo solvent dyes. Although the assumption is that these substances are behaving as single molecules, it would be expected that solid particles would be even more persistent than indicated by the modelled results, which indicated that these substances would be persistent in water, sediment and soil.

When possible, available data from relevant analogues were used to inform the read-across with respect to ecotoxicity. Different formulations or impurities in the Azo Solvent Dyes used in aquatic toxicity tests can affect the outcome of the test results, particularly if a carrier was required for those substances with negligible water solubility. While the soil and sediment exposure media may be important for Azo Solvent Dyes, the available toxicological information is preliminary at this time.

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 where data was lacking 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 (see Potential to Cause Harm to Human Health section). 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.

7. Potential to Cause Harm to Human Health

The human health assessment for Azo Solvent Dyes focuses on substances that are in commerce based on information received in response to the section 71 survey and/or for which available information indicates potential exposure to the general population of Canada. These substances are Solvent Yellow 77, Solvent Orange 3, Sudan I, Sudan IV and Oil Orange SS. Additionally, based on signficant new information on Solvent Red 1, Solvent Red 3 and Solvent Yellow 18, the previous human health assessments for these substances were updated in the context of the Azo Solvent Dyes assessment. Although Oil Orange SS was imported into Canada (Environment Canada 2006), it was not identified to be present in products available to consumers in the Canadian marketplace.

7.1 Exposure Assessment

7.1.1 Environmental media

Empirical data on concentrations of Azo Solvent Dyes in environmental media in Canada or elsewhere were not identified. Based on a study published in 1982, Solvent Yellow 77 was measured in waste water treatment plant influents and effluents and mud samples from the Coosa River Basin, Georgia, USA (Tincher and Robertson 1982). At the time of the study, the Coosa River Basin and its tributaries carried about 50% of all carpet dyeing wastewater in the United States. Measured concentrations of Solvent Yellow 77 ranged from non-detectable to 436 µg/L in wastewater samples and from 140 to 455 µg/L in mud samples. Based on information submitted in response to Phase 1 of the DSL Inventory Update, Solvent Yellow 77 is not manufactured in Canada, but was imported in amounts between 100 and 1000 kg in 2008 (Environment Canada 2009). It is unlikely that recent industrial activity involving Solvent Yellow 77 in Canada would result in levels comparable to the maximum concentrations previously measured in North America from industrial carpet dyeing activities (Tincher and Roberston 1982). Based on its use information and a quantity of 1000 kg/year, the aquatic PEC was estimated to be 10.8 µg/L which is lower than the previously observed range (refer to the Releases to the Environment section under Ecological Exposure Assessment).

Similar to Solvent Yellow 77, Oil Orange SS and Solvent Red 3 were imported in amounts between 100 and 1000 kg, while import quantities between 1000 and 10 000 kg were reported for Sudan I (Environment Canada 2006, 2009). Due to the low volatility and water solubility of Azo Solvent Dyes, these substances are expected to be adsorbed to soil and sediments when released to the environment. Exposure to Azo Solvent Dyes through drinking water is not expected, due to (i) the likely removal of these substances from the water compartment, (ii) the low import quantities of Solvent Yellow 77, Oil Orange SS, Solvent Red 3 and Sudan I and (iii) the absence of major import and manufacturing activities for the remaining Azo Solvent Dyes in Canada. Due to the low vapour pressures of these Azo Solvent Dyes (see Table 3-1 and Table 3-2), inhalation of the volatile fraction is not expected to be a significant route of exposure via

air (refer to the Environmental Fate and Behaviour section). Thus, exposure to the Azo Solvent Dyes through environmental media is not expected.

7.1.2 Food

None of the substances in this Screening Assessment are listed on the *List of Permitted Colouring Agents* in Canada as permitted food colouring agents (Health Canada 2012). Sudan I may be considered a subsidiary dye of Sunset Yellow (E110) if found at very low levels (2011 email from the Food Directorate, Health Canada, to the Risk Management Bureau, Health Canada; unreferenced). In a 2005 summary report published by the Food Standards Agency in the United Kingdom (UK) (Food Standards Agency 2005), Sudan dyes were found in approximately 1% (65 out of 4806) of the food samples collected; types of food samples included oils and fats, soups, broths, sauces, herbs and spices. Based on notifications in the European Commission's Rapid Alert System for Food and Feed, which reported over 10,000 notifications from 2010 to 2012, 34 notifications of Sudan I and 10 notifications of Sudan IV in spices and palm oil products were made in the same two year timeframe (RASFF 2012).

The Canadian Food Inspection Agency (CFIA) has tested for the presence of synthetic food colourants in foods, including seven substances within the Azo Solvent Dyes subgroup: Sudan I, Solvent Orange 7, Solvent Red 23, Sudan IV, Solvent Yellow 2, Oil Orange SS and Solvent Red 19. In the recent 2009-2010 and 2010-2011 targeted food surveys, CFIA analyzed domestic and imported food samples, including spices, which were selected for their high likelihood of containing food colouring agents (CFIA 2010, 2011). None of these seven substances were detected in the earlier survey which tested 100 food samples. In the later survey which tested 1546 food samples, one palm oil was found to contain Sudan IV and one curry-based powder/mix was found to contain Sudan I and Sudan IV. Both products were immediately subjected to a recall. Considering that none of these dyes are permitted for use in or on foods in Canada, the low prevalence of Sudan I and IV dyes found in Canadian targeted food surveys and the absence of remaining Azo Solvent Dyes in tested food samples, exposure to these substances from food in Canada is not expected.

While Sudan IV was identified for use in food packaging applications in Canada as a component of dyes that may be used in resins to package food, minimal potential for direct food contact is expected in this application. Therefore, exposure potential is not expected to be significant (2011 emails from the Food Directorate, Health Canada, to the Risk Management Bureau, Health Canada; unreferenced).

Overall, exposure to Azo Solvent Dyes from foods and food packaging applications is not expected to be significant.

7.1.3 Products

Uses of Azo Solvent Dyes in products that may result in exposure of the general population of Canada include uses in cosmetic products, textiles, leather, shoe polish, writing ink and paper.

Based on notifications submitted under the *Cosmetic Regulations* to Health Canada, Solvent Red 1, Solvent Red 3 and Solvent Yellow 18 are used in certain cosmetic products in Canada (2011 and 2013 emails from the Consumer Product Safety Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada; unreferenced). These cosmetic products and the associated estimated exposures via the dermal and inhalation routes are summarized in Table 7-1; the assumptions and details for these exposure estimates are presented in Appendix G. Exposures to Solvent Red 1 from the use of body moisturizer, hair gel, bath salts, semi-permanent cosmetic tattoo ink and lipstick are not expected, as these uses are no longer present in Canada.

Table 7-1. Summary of estimates of exposure to Solvent Red 1, Solvent Red 3 and Solvent Yellow 18 via use of cosmetic products[a]

Azo Solvent Dye	Cosmetic exposure scenario[b]	Exposure route	Concentration (% w/w)[c]	Estimated daily exposure (mg/kg-bw per day)
Solvent Red 1	Hair conditioner	Dermal	≤ 0.1	5.3×10^{-4}
Solvent Red 1	Hair straightener[d]	Dermal	≤ 0.1	4.9×10^{-4}
Solvent Red 1	Soap, solid form: showering	Dermal	≤ 0.3	2.3×10^{-4}
Solvent Red 1	Manicure preparation gel[d]	Dermal	≤ 0.1	7.8×10^{-5}
Solvent Red 3	Spray perfume	Dermal	≤ 0.1	2.1×10^{-3}
Solvent Red 3	Spray perfume	Inhalation	≤ 0.1	1.2×10^{-6}
Solvent Red 3	Essential oil: massage[d]	Dermal	≤ 0.1	1.9×10^{-3}
Solvent Red 3	Depilatory cream[d]	Dermal	≤ 0.3	2.8×10^{-4}
Solvent Red 3	Soap, liquid form: showering	Dermal	≤ 0.1	9.6×10^{-5}
Solvent Yellow 18	Leave-in hair conditioner	Dermal	≤ 0.1	5.3×10^{-3}
Solvent Yellow 18	Hair conditioner	Dermal	≤ 0.1	5.3×10^{-4}
Solvent Yellow 18	Soap, solid form: showering	Dermal	≤ 0.1	7.7×10^{-5}

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

[a] Exposure estimates are calculated using ConsExpov4.1 (ConsExpo 2006) unless otherwise specified. Refer to Appendix G for exposure factors.

[b] Exposure scenarios consider adults 20–59 years of age. Dermal absorption is assumed to be 26% (Collier et al. 1993).

[c] Based on notifications submitted under the *Cosmetic Regulations* to Health Canada (2011 and 2013 emails from the Consumer Product Safety Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada; unreferenced).

[d] For products that have a use frequency of less than once a day (e.g., depilatory cream: 17 times per year), the estimated daily exposure derives from one year's use amortized over 365 days.

In the absence of data on the dermal absorption of Solvent Red 1, Solvent Red 3 and Solvent Yellow 18, exposures via the dermal route for these three substances were estimated using a dermal absorption of 26% based on an *in vitro* dermal absorption

study of a structurally similar substance Sudan I (Collier et al. 1993). In this study, 26% of the applied dose of Sudan I was absorbed by human skin within 24 hours; this percentage, corresponding to 5 µg/cm^2, includes Sudan I found in both the skin sample and in the receptor fluid. The use of dermal absorption equal to 26% is conservative since the vehicle used in the study is acetone which enhances absorption.

7.1.3.1 p-Aminoazobenzene

p-Aminoazobenzene is one of the EU22 aromatic amines. Despite European Union (EU) legislation and associated restrictions, the presence of some EU22 aromatic amines in certain consumer products originating from one European country and non-European countries has been reported when above 30 ppm. Product monitoring data in Europe from the EU RAPEX database (2010–2012) indicated the presence of *p*-aminoazobenzene in about 35 textile, clothing and leather products (RAPEX 2012). *p*-Aminoazobenzene was found at detectable concentrations ranging up to 1701 mg/kg based on standardized test methods. This substance was present either as a breakdown product of dyes or as a residual from use as a chemical intermediate in the dyeing process. Reported products include clothing, handbags, bed linen, boots, gloves, toys and clothing for children; 27 products were originally manufactured in China, 1 product was manufactured in India, another product was manufactured in France, and the country of origin is unknown for the remaining 6 products (RAPEX 2012).

The information above indicates that *p*-aminoazobenzene is present in presumably a small percentage of imported products on European markets. Given that the Canadian textile market is composed predominantly of imported products (Industry Canada 2011; Environment Canada and Health Canada 2013b), together with the fact that *p*-aminoazobenzene is still detected in some imported textiles and leather products in Europe, despite existing restrictions, the potential for this substance to be present in a small percentage of imported products in Canada is recognized.

Results from product testing by Health Canada did not indicate the presence of *p*-aminoazobenzene in textile and leather products in Canada (Health Canada 2013a). These results are consistent with the global phase out of azo dyes based on the EU22 aromatic amines due to restrictions in other countries (Environment Canada and Health Canada 2013a). Combined with the lack of manufacturing and import activities reported in response to section 71 surveys for *p*-aminoazobenzene, significant exposure of the general population of Canada is not expected.

7.1.3.2 Solvent Yellow 77

The use of Solvent Yellow 77 in textiles and leather has been reported (Tincher and Robertson 1982; IARC 1990). Upper-bounding daily exposures to Solvent Yellow 77 from textile products were estimated to range from 5.5×10^{-4} to 8.7×10^{-4} mg/kg of body weight (kg-bw) per day via the dermal route from direct skin contact with personal apparel; upper-bounding exposure estimates from direct skin contact with leather products range from 4.4×10^{-4} to 1.7×10^{-2} mg/kg-bw per event (see Appendix G for

more details). The estimated exposures are based on a dermal absorption of 21.6% reported in an *in vivo* study on Solvent Yellow 2 (Feldmann and Maibach 1970). Solvent Yellow 2 and Solvent Yellow 77 are both azobenzene derivatives that are structurally similar and have similar water solubilities and log K_{ow} values; the water solubility of Solvent Yellow 77 is 1.18 mg/L (Baughman and Perenich 1988b) whereas that of Solvent Yellow 2 ranges from 0.22 to 1.4 mg/L (refer to Table A2-1), the log K_{ow} value of Solvent Yellow 77 was reported to be 3.6 (Sigma-Aldrich 2010) whereas that of Solvent Yellow 2 was reported to be 4.58 (NTP 2011) (refer to Appendix B). Based on structural similarity and similar physical-chemical properties, dermal absorption value of 21.6% from Solvent Yellow 2 was applied to Solvent Yellow 77. The use of dermal absorption equal to 21.6% is conservative since the vehicle used in the *in vivo* study is acetone which enhances absorption.

Oral exposure resulting from mouthing of textiles by infants was estimated to be 2.7×10^{-5} mg/kg-bw per day as an upper-bounding estimate (see Appendix G for more details).

Solvent Yellow 77 was also identified to be used as a dye in pulp and paper manufacturing in the United States (Scorecard 2011). 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 Solvent Yellow 77 from paper products are unlikely due to the physical and chemical properties of Solvent Yellow 77 as well as the impregnation of the colourant in the paper.

7.1.3.3 Solvent Orange 3

Solvent Orange 3 was identified as an ingredient in 2 common shoe polish products in the United States (HPDB 2012), which are also available on the Canadian market. Per event dermal exposure to Solvent Orange 3 via the use of shoe polish was estimated to be 0.021 mg/kg-bw (see Appendix G).

7.1.3.4 Sudan I

Although no information was received from the section 71 survey regarding the presence of Sudan I in pen ink sold in Canada, the use of this substance in ballpoint pen inks appears to be a common one, based on information in the jointly published Colour Index International database of the Society of Dyers and Colourists and the American Association of Textile Chemists and Colorists (CII 2011). The per event dermal exposure to Sudan I from ballpoint pen ink was estimated to range from 4.2×10^{-4} to 2.1×10^{-3} mg/kg-bw in 0.5- to 4-year-olds based on a dermal absorption of 26% (Collier et al. 1993). Incidental per event oral exposure to Sudan I in ink via hand-to-mouth behaviours was conservatively estimated to range from 1.6×10^{-3} to 8.1×10^{-3} mg/kg-bw in the same age group. These estimates are considered to be upper-bounding due to the conservative assumptions used in the exposure scenario (see Appendix G). Sudan I was also reported to be present in approximately 70 children's

products, including plastic toys, clothing and textile furnishings, on the American market according to data submitted by consumer companies to the Washington State Department of Ecology from October 2011 to July 2014 under the *Children's Safe Products Act* (Washington State Department of Ecology 2014). No information was identified regarding the presence of Sudan I in children's products in Canada. However, given the similarity between the American consumer market to that of Canada, the potential for Sudan I to be present in a limited number of similar products in Canada is recognised. If present, exposure to Sudan I from such reported children's products is expected to be minimal compared with that from writing ink, since the substance in plastic toys would be encapsulated in a plastic matrix and the substance in textiles is used at relatively low concentrations (i.e., less than 500 mg of dye per kg of textile).

7.1.4 Uncertainty

There is uncertainty in the characterization of exposure to Azo Solvent Dyes from foods and food packaging applications. Regulatory agencies both abroad and domestically monitor for the Sudan dyes and do occasionally find foods to which the dyes have been illegally added (Food Standards Agency 2005; CFIA 2010, 2011; RASFF 2012). However, the presence of the dyes in foods is overall very low and dietary exposure is considered to be low. In Canada, the CFIA is responsible for monitoring the food supply for the presence of non-approved food colours.

The reports on *p*-aminoazobenzene in the EU RAPEX database (2010-2012) is specific primarily to imported textile and leather products in the European market. Uncertainty in characterizing exposure to this substance based on the results of the Canadian market survey of the limited number of products is acknowledged (Health Canada 2013a).

There is also uncertainty associated with the presence of Azo Solvent Dyes in imported products in Canada due to the limited Canadian information available on imported products in general.

Exposure scenarios presented in this report are considered to represent the predominant sources of exposure based on use patterns and are based on conservative assumptions (see Appendix G). Exposures to Solvent Yellow 77 from textiles presented in this Screening Assessment are likely overestimates. For example, the use of Solvent Yellow 77 in textile dyeing processes likely results in the encapsulation of the dye in the textile fibre polymer, thereby minimizing its leaching from the final product. However, it is unknown to what extent this occurs and therefore the effect of encapsulation in the textile fibre is not factored into the estimation of exposure. It is not expected that Solvent Yellow 77 would be present in 100% of consumer products made of textiles in Canada. Therefore, exposures were estimated assuming, based on professional judgement, that there is a 10% probability that this substance is used in dyeing products made of textile in Canada. This adjustment factor 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 G for further explanation.

Because there are limited dermal absorption studies for this Azo Solvent Dyes subgroup, available data were used for read-across to other substances in this assessment. There is a higher confidence in read-across of dermal absorption data from the *in vitro* study on Sudan I (Collier et al. 1993) to Solvent Red 1 and Solvent Red 3 than to Solvent Yellow 18. Sudan I, Solvent Red 1 and Solvent Red 3 have the same structural backbone, consisting of an azo bond between a naphthyl ring and a phenyl ring, and the only differences between these two substances and Sudan I is one or two substituents: an additional methoxy (CH3O-) group in Solvent Red 1, an additional ethoxy (CH3CH2O-) group in Solvent Red 3 and the para position of the hydroxyl (-OH) group instead of being ortho as in Sudan I. The ortho-OH in Sudan I allows for hydrogen bonding to the azo linkage, which is not possible in Solvent Red 3. The effect of this difference on dermal absorption is unknown. There is also uncertainty associated with the use of acetone as the vehicle used in the *in vitro* dermal absorption study by Collier et al (1993); acetone is known to enhance absorption through the skin.

There is uncertainty in the dermal absorption of Solvent Yellow 77 due to extrapolation of the results of an *in vivo* study on Solvent Yellow 2 (Feldmann and Maibach 1970), although the two substances are structurally similar and are in the same subset (see Appendix A). Uncertainty exists also because of the use of acetone as a vehicle, which may have enhanced absorption of the substance through skin. In addition, the percentage of dermal absorption was corrected for incomplete renal excretion based on 14C recovery in urine after intravenous administration in guinea pigs rather than humans.

While there were *in vitro* dermal absorption studies reporting mean absorption rates for Solvent Yellow 77 using human epidermis (ETAD 1994, 1995), these studies were not applied in this assessment due to the uncertainty associated with the lack of reporting of the skin-bound portion of the substance.

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 2013a).

Azo Solvent Dyes are bioavailable to varying extents based on empirical data. Some of them possess free aromatic amines that are readily metabolized to electrophilic intermediates without azo bond cleavage. Reductive cleavage of azo bonds was also observed in Azo Solvent Dyes in experiments *in vivo* and *in vitro*. However, the extent of cleavage varies among individual dyes, depending on their physical and chemical properties, such as solubility and molecular size. Additionally, oxidative cleavage of azo bonds, which releases benzenediazonium and other reactive electrophilic metabolites, was also observed or expected in some Azo Solvent Dyes.

In the following sections, the critical health effects, including carcinogenicity, genotoxicity and other health effects are evaluated for individual substances in each of the three health subsets: Azobenzene and Its Derivatives, Sudan Dyes and Miscellaneous Substances. Information on absorption, distribution, metabolism and excretion is provided where data are available. Data availability varied significantly from substance to substance for Azo Solvent Dyes. When only limited empirical data were identified for a target substance, additional health information from structurally similar substances and/or metabolites is also considered. In addition, the critical health effects for Solvent Red 1, Solvent Red 3 and Solvent Yellow 18, which were not fully characterized in the previous assessment report under the Challenge Initiative, are reviewed in this assessment.

7.2.1 Azobenzene and Its Derivatives

The health effects assessment for Azobenzene and Its Derivatives (Table 7-2) is conducted based on the empirical data on individual substances. Azo bond reductive cleavage can activate or reduce the toxicity of the substances in this health subset, depending on their structures.

Table 7-2. Azobenzene and Its Derivatives

Substance name (CAS RN)	Postulated azo bond reductive cleavage products (CAS RN)
Azobenzene (103-33-3)	Aniline (62-53-3)
p-Aminoazobenzene (60-09-3) [EU 22 aromatic amine]	Aniline (62-53-3) *p*-Phenylenediamine (106-50-3)
Solvent Yellow 2 (60-11-7)	Aniline (62-53-3) *N*,*N*-Dimethyl-*p*-benzenediamine (99-98-9)
Solvent Orange 3 (495-54-5)	Aniline (62-53-3) 1,2,4-Triaminobenzene (615-71-4)
Solvent Yellow 3 (97-56-3) [EU22 aromatic amine]	2-Methyl-1,4-benzenediamine (95-70-5) *o*-Toluidine (95-53-4) [EU22 aromatic amine]
Solvent Yellow 77 (2832-40-8)	*p*-Aminoacetanilide (122-80-5) 2-Amino-4-methylphenol (95-84-1)

Abbreviation: CAS RN, Chemical Abstracts Service Registry Number

7.2.1.1 Azobenzene (CAS RN 103-33-3)

7.2.1.1.1 Absorption, distribution, metabolism and excretion

When rats were given azobenzene orally, aniline and hydrazobenzene were detected in the conventional rats, but aniline was not detected in the germ-free rats (Macholz et al. 1985), indicating that the azo bond reductive cleavage of azobenzene was mainly mediated by the gut microflora. Following intraperitoneal injection of azobenzene, aniline and benzidine[7] were detected in the urine of the treated rats (Elson and Warren

[7]The authors considered that benzidine might be converted from hydrazobenzene via a non-enzymatic reaction under acidic conditions.

1944). When pregnant rats received azobenzene by gavage during gestation, azobenzene and hydrazobenzene were detected in the placentas and the fetuses (Kujawa et al. 1985). A study in rabbits (route not specified) reported that 30% of dosed azobenzene was found in feces, and various metabolites of azobenzene (with intact azo bond) and aniline and their conjugates, as well as hydrazobenzene and benzidine, were detected in the urine (Bray et al. 1951). In addition, cytochrome P450 (CYP) enzyme levels and activities were increased in the rat liver following intraperitoneal injection (Rinkus and Legator 1979; Fujita et al. 1984; Cheung et al. 1994); however, decreased liver cytochrome P450 contents were observed in rats following subcutaneous injection (Wisniewska-Knypl 1978). It was reported that the azo bond of azobenzene was reduced *in vitro* by bovine liver enzymes (Kim and Shin 2000) and rabbit liver enzymes (Fouts et al. 1957).

7.2.1.1.2 Carcinogenicity and genotoxicity

Azobenzene has been classified by the European Commission as a Category 1B carcinogen – “May cause cancer” and as a Category 2 mutagen – “Suspected of causing genetic defects” (European Commission 2008) and by the US Environmental Protection Agency (US EPA) as a Group B2 substance – “Probable human carcinogen” (US EPA 1993). It was classified by the International Agency for Research on Cancer (IARC) as a Group 3 substance – “Not classifiable as to its carcinogenicity to humans” (IARC 1987a).

The carcinogenic potential of azobenzene has been investigated in rats and mice via oral administration. F344 rats fed 200 or 400 mg/kg of azobenzene in the diet (10 or 20 mg/kg-bw per day) for 105 or 106 weeks showed increased incidences of sarcomas, including fibrosarcomas, hemangiosarcomas and osteosarcomas in both sexes of the treated rats and malignant hemangiopericytomas in the treated female rats in the spleen and other abdominal organs. The results were statistically significant at the higher dose level (NCI 1979; Goodman et al. 1984). Tumour incidences were not significantly increased in B6C3F1 mice treated in the same study, in which male mice were fed 200 or 400 mg/kg of azobenzene in the diet (26 or 52 mg/kg-bw per day) for 105 weeks and female mice were fed 400 or 800 mg/kg of azobenzene in the diet (52 or 104 mg/kg-bw per day) for 38 weeks followed by feeding 100 and 400 mg/kg of azobenzene in the diet (13 and 52 mg/kg-bw per day) for 67 or 68 weeks (NCI 1979). When newborn mice received 21.5 mg/kg-bw per day of azobenzene by stomach tube from 7 to 28 days of age followed by 56 mg/kg in the diet (7.28 mg/kg-bw per day) for 80 weeks, significantly increased incidences of liver tumours were observed only in the treated male (C57BL/6 × C3H/Anf)F_1 mice; tumours were not observed in the treated female (C57BL/6 × C3H/Anf)F_1 mice or either sex of the treated (C57BL/6 × AKR)F_1 mice (IARC 1975c). Lung tumours were observed in A/J mice 24 weeks after receiving azobenzene by gavage for 8 weeks (total dose of 300 mg/kg-bw, 5.4 mg/kg-bw per day) (Stoner et al. 1986).

Azobenzene did not exhibit tumour initiating ability in SENCAR mice (Bull et al. 1986). Additional cancer bioassays conducted via a single subcutaneous injection (1000

mg/kg-bw) in (C57BL/6 × C3H/Anf)F_1 and (C57BL/6 × AKR)F_1 mice (IARC 1975c), repeated intraperitoneal injections (total dose of 60, 150 or 300 mg/kg-bw) in A/J mice (Stoner et al. 1986) or repeated subcutaneous injections (total dose of 3.1 g, lifetime exposure) in Sherman rats (Spitz et al. 1950) did not provide clear evidence of azobenzene-induced tumour formation.

The genotoxic potential of azobenzene has been investigated extensively *in vivo* and *in vitro*. Azobenzene caused chromosomal and deoxyribonucleic acid (DNA) damage *in vivo*. Positive micronucleus assay results were observed in the bone marrow and peripheral blood of orally dosed rats and in the bone marrow of orally dosed mice (weakly positive) (George et al. 1990; Wakata et al. 1998). Azobenzene caused DNA damages (comet assays) in the stomach, colon, urinary bladder and lung of orally dosed mice, in the stomach, colon, liver, kidney, urinary bladder, lung and bone marrow of orally dosed rats and in the mouse stomach, liver and lung following intraperitoneal injection (Tsuda et al. 2000; Sekihashi et al. 2001, 2002). In *Drosophila*, azobenzene induced chromosome recombination following oral administration (Vogel and Nivard 1993), but not gene mutation in the sex-linked recessive lethal assay via oral dosing or adult injection (Zimmering et al. 1985).

In vitro, azobenzene showed some mutagenicity in bacteria. Positive Ames test results were repeatedly observed in *Salmonella typhimurium* strain TA100 upon metabolic activation (McCann et al. 1975; Haworth et al. 1983; Mori et al. 1986; Zeiger 1987; NTP 1988a; Cheung et al. 1994); the Ames test results were mainly negative in strain TA100 without metabolic activation and negative in strains TA98, TA1535 and TA1537 with and without metabolic activation (Haworth et al. 1983; Mori et al. 1986; Zeiger 1987). Azobenzene also induced gene mutations in a photobacterium (Xie et al. 1999). Azobenzene induced sister chromatid exchanges in Chinese hamster ovary (CHO) cells with metabolic activation (Galloway et al. 1985), whereas the results of chromosomal aberration assays in CHO cells were inconclusive (Galloway et al. 1985; NTP undated-a). Azobenzene caused DNA strand breaks (Sina et al. 1983; Storer et al. 1996), but not unscheduled DNA synthesis, in primary rat hepatocytes (Mori et al. 1986). Azobenzene induced DNA damage/repair in the *uvrB/recA* assay in *Escherichia coli* k-12 without metabolic activation (Hellmér and Bolcsfoldi 1992a). In the presence of metabolic activation, the *recA* assay results were negative (Suter and Jaeger 1982; Hellmér and Bolcsfoldi 1992a). In the host-mediated DNA damage/repair assays, weakly positive results were observed following intraperitoneal injection, and the results were negative following oral administration (Hellmér and Bolcsfoldi 1992b). Azobenzene induced a weak SOS response in *S. typhimurium* TA1535/pSK1002 in *umu* tests with metabolic activation (Nakamura et al. 1987). It inhibited DNA repair processes in human lymphocytes after ultraviolet (UV) irradiation (Gaudin et al. 1971).

Azobenzene did not induce cell transformation in the C3H/10T1/2 mouse embryo fibroblast cell line (Kowalski et al. 2001).

7.2.1.1.3 Other health effects

In the aforementioned 2-year dietary studies in rats and mice (NCI 1979), deposits of hemosiderin in the spleen, liver and renal tubular epithelium of the treated female rats and chronic capsulitis of the spleen in all treated rats (10 or 20 mg/kg-bw per day) were observed. Enlarged spleens and mesenteric nodes were observed in a short-term dietary testing (7 weeks) in the treated mice at 700 – 4600 ppm (91 –598 mg/kg-bw per day) dose levels, whereas minor renal and liver injuries were noted at 1000 – 2000 ppm (50 –100 mg/kg-bw per day) dose levels in the treated rats (NCI 1979). In another short-term oral study, liver damage and mortality were observed in dogs fed 600 ppm of azobenzene in the diet (18 mg/kg-bw per day) for 63 days (Gosselin et al. 1984).

7.2.1.1.4 Summary

Overall, azobenzene exhibited carcinogenicity and some genotoxicity and elicited haematological effects in experimental animals. It caused chromosomal damages *in vivo* and DNA damages *in vivo* and *in vitro*, but it only showed a weak mutagenicity in bacteria. Azobenzene induced spleen tumours in both sexes of orally dosed rats, and the precursor events featured with hemosiderosis and capsulitis, likely through a pathway similar to that of aniline, i.e., “injury to erythrocytes and scavenging of these chemically damaged erythrocytes by the spleen produces an iron overload or oxidative damage to macromolecules, which may result in a carcinogenic response in the spleen” (Health Canada 2011). Azobenzene induced liver and lung tumours in mice, in a strain- and sex-specific manner, for which the modes of action have not been fully elucidated.

7.2.1.2 *p*-Aminoazobenzene (CAS RN 60-09-3)

7.2.1.2.1 Absorption, distribution, metabolism and excretion

When rats received *p*-aminoazobenzene orally, sulfate conjugates of hydroxy-4-aminoazobenzene (4′-, 3′- and 3,4′-), *N*-acetyl-4′-hydroxy-4-aminobenzene and *p*-acetamidoacetanilide as well as conjugated forms of *p*- and *o*-aminophenol were detected in the urine of the rats (Ishidate and Hashimoto 1962). Following intraperitoneal injection of *p*-aminoazobenzene, hydroxyl- and acetyl- metabolites of *p*-aminoazobenzene (with an intact azo bond) were detected in the urine of the rats (Sato et al. 1966). Robinson et al. (1964) reported that almost 100% of the expected *p*-aminophenol and only about 20–30% of the expected *p*-phenylenediamine were recovered from the urine of the rats following intraperitoneal injection of 10 mg of ^{14}C-labelled *p*-aminoazobenzene. It has been suggested that *N*-sulfoöxy-4-aminoazobenzene was the major ultimate electrophilic metabolite that led to liver tumour development in mice (Delclos et al. 1986). In addition, it was reported that the azo linkage of *p*-aminoazobenzene was reduced by intestinal bacteria (BUA 2000).

7.2.1.2.2 Carcinogenicity and genotoxicity

p-Aminoazobenzene has been classified by IARC as a Group 2B substance – “Possibly carcinogenic to humans” (IARC 1987a) and by the European Commission as a

Category 1B carcinogen – “May cause cancer” (European Commission 2008). *p*-Aminoazobenzene is one of the 22 aromatic amines (EU22) that have been classified as carcinogenic in the EU (EU 2006).

The carcinogenic potential of *p*-aminoazobenzene has been investigated in experimental studies in rats and mice via oral administration, in rats via dermal application and in rats and mice via subcutaneous and intraperitoneal injection. These studies did not follow current standard cancer bioassay protocols. Most of the studies have been reviewed by IARC (1975a).

When male Wistar rats were fed a low protein diet containing 0.2–1% *p*-aminoazobenzene over 2 years, significantly increased incidences of liver tumours, including hepatomas and carcinomas, were observed; however, it was also noticed that *p*-aminoazobenzene-induced liver damages was influenced by the dietary constituents (Kirby 1947; Kirby and Peacock 1947). In several other studies that used lower oral doses of *p*-aminoazobenzene in Donryu, SD and albino rats, no evidence of liver tumours was observed (Sasaki and Yoshida 1935; Miller and Miller 1948; Odashima and Hashimoto 1968; Delclos et al. 1986). When female CD-1 mice were fed 0.035% *p*-aminoazobenzene in the diet (45.5 mg/kg-bw per day) for 10 months, 76% of the treated mice developed liver tumours, but none in the low-dose (23.4 mg/kg-bw per day) and the control groups (Delclos et al. 1986). It was noticed that the liver tumour incidences in mice were dramatically reduced in the presence of the sulfotransferase inhibitor, pentachlorophenol, indicating the importance of sulfonation for the activation of *p*-aminoazobenzene (Delclos et al. 1986).

Skin tumours (benign or malignant) were observed in all six treated male stock albino rats after skin painting (uncovered) of 1 mL of a 0.2% *p*-aminoazobenzene solution in acetone, twice weekly for life. No dye stained liver or liver lesions were observed (Fare 1966).

In addition, newborn mice (strains B6C3F1, C3H/He, C57BL/6 and CD-1) received *p*-aminoazobenzene (≥ 3.5 mg/kg-bw per injection) by either a single intraperitoneal injection on day 12 or four injections on days 1, 8, 15 and 22 developed liver tumours at 10–11 months of age (Miller et al. 1979; Delclos et al. 1984, 1986; Manam et al. 1992b). Male newborn mice were more susceptible to the liver tumour induction. However, when male newborn rats (F344) were treated in a similar way, no liver tumours were observed at 2 years of age (Delclos et al. 1984). Increased lung tumour incidences were observed in CD-1 mice at 9–12 months of age after a single intraperitoneal injection of *p*-aminoazobenzene (approximately 79 mg/kg-bw) on postnatal day 12, but not in those received repeated intraperitoneal injections of *p*-aminoazobenzene at a lower dose (20 mg/kg-bw for 15 days, starting on postnatal day 12) (Manam et al. 1992a; BUA 2000). Newborn mice (strain CDF1) received subcutaneous injection of 98.5 mg/kg-bw of *p*-aminoazobenzene on day 1 followed by three injections at 1-week intervals developed liver tumours and lymphoma or leukemia at 1 year of age in both sexes (Fujii 1983). When female ICR/LCL mice were subcutaneously injected with *p*-aminoazobenzene on gestation days 15, 17 and 19, the combined incidences of liver, hematopoietic and

lymphoid tumours in the offspring of the treated mice were significantly higher than those from the control group (Fujii 1983). No tumours were observed in rats (male CD and Fischer) after subcutaneous injection of low doses of *p*-aminoazobenzene for 12 weeks (Poirier et al. 1967; Miller et al. 1979), nor in stock mice received intermittent subcutaneous injection of *p*-aminoazobenzene (every 2 weeks) for life (Kirby 1945a, b).

The genotoxic potential of *p*-aminoazobenzene has been investigated *in vivo* and *in vitro*. In the cancer bioassays in mice (Manam et al. 1992a, b), gene mutations at Ki-*ras* 13 in the liver and lung tumours and at N-*ras* 12 and 13 in the liver tumours were detected in mice treated with *p*-aminoazobenzene by intraperitoneal injection, but not in those spontaneous tumours. In addition, the mutation frequencies at Ha-*ras* 61 were higher in the liver tumours of the treated mice than that in the spontaneous liver tumours. *p*-Aminoazobenzene aerosol produced gene mutation in *Drosophila* in the sex-linked recessive lethal assay (Demerec 1949). The clastogenicity of *p*-Aminoazobenzene was observed in micronucleus assays in the bone marrow and blood of the treated mice and rats following intraperitoneal injection (Morita et al. 1997a, b; Wakata et al. 1998), in chromosomal aberration assays in the bone marrow of the treated rats (exposure route not specified) (Kawachi et al. 1980a, b) and in sister chromatid exchange assays in the bone marrow of the treated mice following intraperitoneal injection (Parodi et al. 1983). *p*-Aminoazobenzene produced DNA damages (comet assays) in the stomach, colon, lung, brain, liver and bladder of orally dosed mice (Tsuda et al. 2000) and in the liver, kidney and spleen of mice (Sasaki et al. 1997) and in the liver of rats following intraperitoneal injection (Parodi et al. 1981; Brambilla et al. 1985). DNA adducts, *N*-(deoxyguanosin-8-yl)-4-aminoazobenzene, were detected in the liver of B6C3F1 mice and Fischer rats following intraperitoneal injection of *p*-aminoazobenzene (Delclos et al. 1984, 1986).

The mutagenicity of *p*-aminoazobenzene has been extensively investigated in bacteria. With metabolic activation, the majority of the Ames tests results was positive in *S. typhimurium* strains TA98, TA100 and TA1538, but negatvie in strains TA97, TA102, TA104, TA1535, TA1536, TA1537, C3076, D3052 or G46 or in *Escherichia coli* strains WP2 and WP uvrA−. The Ames test results were negative without S9 (Szybalski 1958; Ames et al. 1973; McCann et al. 1975; Yahagi et al. 1975; Commoner 1976; Nagao et al. 1977; Anderson and Styles 1978; Matsushima et al. 1978; Degawa et al. 1979; Rosenkranz and Poirier 1979; Simmon 1979a; Kawachi et al. 1980a; Pienta 1980b; Hashimoto et al. 1981; Parodi et al. 1981; Miyagoshi et al. 1985; Zeiger et al. 1992; Cheung et al. 1994; Lee et al. 1994; BUA 2000; Hakura et al. 2005). In host-mediated gene mutation assays, Swiss-Webster mice were administered *p*-aminoazobenzene via gavage or intramuscular injection, followed by intraperitoneal injection of *S. typhimurium* strain TA1530, TA1538 or *Saccharomyces* D3. Positive results were observed only in strain TA1530 in mice treated by intramuscular injection (Simmon et al. 1979). *p*-Aminoazobenzene also induced gene mutations in mammalian cells in mouse lymphoma assay with metabolic activation (Amacher and Turner 1982), but it did not induce gene mutations in the metabolically competent rat liver cell lines (Tong et al. 1980, 1984). *p*-Aminoazobenzene exhibited clastogenicity *in vitro*. Positive chromosomal aberration and sister chromatid exchange results were observed in

hamster cells with metabolic activation (Kawachi et al. 1980a); without metabolic activation, the sister chromatid exchange results were negative in both rat hepatoma and esophageal tumour cell lines (Abe and Sasaki 1982; Abe 1984a, b). *p*-Aminoazobenzene also caused DNA damage/repair *in vitro*. Positive results of unscheduled DNA synthesis were observed in primary rat and mouse hepatocytes in the majority of the studies, but not in human skin epithelial cells without metabolic activation (Williams 1976, 1977; Lake et al. 1978; Brouns et al. 1979; Probst and Hill 1980; Probst et al. 1981; Watanabe and Hashimoto 1981; Mori et al. 1986). *p*-Aminoazobenzene produced DNA damages in human fibroblasts (Rahimtula et al. 1982) and in the bacteria *Bacillus subtilis* and *E. coli* with metabolic activation (Ichinotsubo et al. 1977; Kada et al. 1980; Kawachi et al. 1980a). Other DNA damage/repair assays in bacteria, including SOS assay in *S. typhimurium* (Shimada et al. 1989) and differentiated cell killing assay in *E. coli* (Rosenkranz and Poirier 1979; Rosenkranz and Leifer 1980), were either negative or inconclusive. *p*-Aminoazobenzene did not induce chromosomal recombination in yeast (Simmon 1979b).

p-Aminoazobenzene induced Syrian hamster embryo cell and baby kidney (BHK21/C13) cell transformation, but not mouse or rat embryo cell transformation, with metabolic activation (Ashby et al. 1978; Poiley et al. 1979; Pienta 1980a, b; Heidelberger et al. 1983).

7.2.1.2.3 Other health effects

Only limited data have been identified with respect to the reproductive and developmental effects of *p*-aminoazobenzene. In a cancer study in pregnant female ICR/LCL mice, increased total tumour incidences in the offspring were observed, indicating that *p*-aminoazobenzene or its metabolites were transferred across the placenta following subcutaneous injection (Fujii 1983). In another study, when female BD rats were fed 10 mg of *p*-aminoazobenzene in the diet (29 mg/kg-bw per day), the estrus of the treated rats was completely inhibited after 7–35 days; the effect was reversed after the treatment ended (Danneberg and Schmähl 1952).

In an old study with limited information (no data on the controls), it was reported that *p*-Aminoazobenzene induced blood effects, including hemolytic anemia, reduced hemoglobin level and anisocytosis, as well as centrilobular hyaline degeneration and pigmented Kupffer cells in the liver and hemosiderin pigmentation in the spleen, kidney and liver in rats fed 0.1% *p*-aminoazobenzene (50 mg/kg-bw per day) in a diet that consists of 4% or 27% casein for 35–41 days; these effects were severer in rats fed the lower protein diet (4% casein) (Smith et al. 1943). Following a single dermal application of 2000 mg/kg-bw of *p*-aminoazobenzene for 24 hours, slight cyanosis was observed in one of the five treated male rats and two of the five treated female rats (BUA 2000). Additionally, Wistar rats exposed to *p*-aminoazobenzene aerosol in acetone polyethylene glycol (1:1) at 0.023–2.8 mg/L for 4 hours had dose-related increases in methemoglobin concentrations. Clinical symptoms, including bradypnea, cyanosis and reduced body temperature, were observed in the higher dose groups (Bayer AG 1993).

p-Aminoazobenzene caused skin sensitization in guinea pigs (Xie et al., 2000) and in many human cases (BUA 2000; Wilkinson and Thomson 2000; Devos and van der Valk 2001; Francalanci et al. 2001; Uter et al. 2001, 2002; Giusti et al. 2002, 2003; Seidenari et al. 2002; Søsted et al. 2002, 2004; Giusti and Seidenari 2003; Trattner et al. 2003; Geier et al. 2004; Nardelli et al. 2004, 2005; Holden and Gawkrodger 2005; Valks et al. 2005; Aalto-Korte et al. 2007; Hillen et al. 2007).

7.2.1.2.4 Summary

Overall, *p*-Aminoazobenzene exhibited carcinogenicity and genotoxicity and elicited haematological effects in experimental animals. There is sufficient *in vivo* and *in vitro* evidence indicating that *p*-aminoazobenzene is mutagenic and clastogenic. It also caused DNA damages *in vivo* and *in vitro*. *p*-Aminoazobenzene induced liver tumours via oral intake in rats and mice and skin tumours via dermal application in rats. Following intraperitoneal injection, gene mutations in the liver and lung tumours and DNA adducts in the livers of the treated rats and mice were detected. It is possible that *p*-aminoazobenzene could induce similar health effects through oral intake as similar metabolites were detected via both routes of exposure. Reactive metabolites of *p*-aminoazobenzene could also be generated by skin metabolism or microbe-mediated azo bond cleavage; however, data for the dermal route of exposure are limited.

Aryl amines are well known to induce methemoglobin formation via aryl hydroxylamines (Lin and Wu 1973; BUA 2000; Neumann 2005). Aryl hydroxylamines can be generated from *N*-hydroxylation of *p*-aminoazobenzene or its azo bond cleavage products, 1,4-benzenediamine and aniline.

7.2.1.3 Solvent Yellow 2 (CAS RN 60-11-7)

7.2.1.3.1 Absorption, distribution, metabolism and excretion

Solvent Yellow 2 is primarily metabolized in the liver (IARC 1975f). When mice were given Solvent Yellow 2 in the diet (165 mg/kg-bw per day) for 7–120 days, significantly increased lipid peroxidation and enzyme (aspartate aminotransferase, alanine aminotransferase, acid phosphatase and alkaline phosphatase) activities were observed (Biswas and Khuda-Bukhsh 2005). Several dimethyl- and hydroxyl-metabolites of Solvent Yellow 2 (with an intact azo bond) and their conjugates have been detected in rats following oral administration, intravenous injection and intraperitoneal injection (Miller et al. 1945; Matsumoto and Terayama 1961; Scribner et al. 1965; Sato et al. 1966; Coles et al. 1983; Samuels et al. 1983). Solvent Yellow 2 and its metabolites were found to bind to liver protein, DNA and ribonucleic acid (RNA) (Roberts and Warwick 1966a, b). The metabolic activation of Solvent Yellow 2 in the liver of mice and rats includes *N*-demethylation (Solvent Yellow 2 to monomethylaminoazobenzene [MAB] and aminoazobenzene [AB]), *N*-hydroxylation of MAB and AB, esterification of *N*-hydroxy-MAB and *N*-hydroxy-AB, and covalent binding of the ultimate metabolites to DNA to form DNA adducts (Delclos et al. 1984). Solvent Yellow 2 also underwent azo bond reductive cleavage *in vivo* and *in vitro*. Its azo bond reductive products, including *p*-aminophenol, *N*-acetyl-*p*-aminophenol, *p*-

phenylenediamine and *N*,*N*′-diacetyl-p-phenylenediamine, were detected in rats following oral administration or intraperitoneal injection (Stevenson et al. 1942; Robinson et al. 1964). Its azo linkage was reduced *in vitro* by rat liver homogenates (Mueller and Miller 1949) and yeast (IARC 1975f). It was also observed that the metabolism of Solvent Yellow 2 was influenced by the animal diet and nutrition (IARC 1975f). The dermal absorption of Solvent Yellow 2 has been investigated *in vivo*; upon topical application for 5 days, the total recovered applied dose (in acetone) from urine ranged from 21.6% in humans to 100% in rabbits (Bartek et al. 1972).

7.2.1.3.2 Carcinogenicity and genotoxicity

Solvent Yellow 2 has been classified by IARC as a Group 2B substance – "Possibly carcinogenic to humans" (IARC 1987a), by the European Commission as a Category 2 carcinogen – "Suspected of causing cancer" and a Category 2 mutagen – "Suspected of causing genetic defects" (European Commission 2008) and by the US National Toxicology Program (US NTP) as "Reasonably anticipated to be a human carcinogen" (NTP 2011).

The carcinogenic potential of Solvent Yellow 2 has been investigated in rats, mice, dogs, monkeys, hamsters and guinea pigs via oral administration, in rats and mice via dermal application and in rats and mice via subcutaneous or intraperitoneal injection. Most of the studies were reviewed by IARC (1975f).

Increased liver tumour incidences were observed in orally dosed rats (Wistar, Sprague-Dawley and unknown strains) in several studies (Kinosita 1936, 1937; Kirby and Peacock 1947; Druckrey and Küpfmüller 1948; Druckrey 1967; Lin and Wu 1974; Delclos et al. 1986). These studies did not follow current standard cancer bioassay protocols, and some of the reports have only limited information or information from a secondary source. The induction time of liver tumours in rats was reported to be inversely proportional to the daily doses, ranging from 34 days at 85.7 mg/kg-bw per day dose level to 700 days at 2.86 mg/kg-bw per day dose level, whereas at dose levels less than 1 mg/kg-bw per day for lifetime, no liver tumours were observed (IARC 1975f). It was reported in a study, which has not been reviewed by IARC (1975f), that significantly increased hepatoma incidences were observed in both sexes of Wistar rats fed a low-protein diet containing 600–1000 mg/kg of Solvent Yellow 2 for up to 33 weeks (Kirby and Peacock 1947). Solvent Yellow 2 also induced liver nodules or tumours in orally dosed mice, including female CD-1, Swiss albino, male C3H/HeOs, female strain C (weak evidence), male CF1, C57BL and heterogeneous type B mice that were fed 26 mg/kg-bw per day or more of Solvent Yellow 2 in the diet (Andervont 1944; Waterman and Lignac 1958; Akamatsu and Ikegami 1968; IARC 1975f; Delclos et al. 1986; Caballero et al. 2004; Biswas and Khuda-Bukhsh 2005).

Increased lung tumour incidences were observed in albino stock mice fed 150 mg/kg or more of Solvent Yellow 2 in the diet (19.5 mg/kg-bw per day) for 75 days to 4 months (Jaffe 1947).

Bladder papillomas with invasion were observed in 2 of the 10 Irish Terriers or mongrel dogs fed 20 mg/kg-bw per day of Solvent Yellow 2 in the diet for 16 months or longer; the rest of the treated dogs died within 16 months. Bladder tumours were not observed in dogs after receiving a lower dose of Solvent Yellow 2 (5 mg/kg-bw per day) for 20 years (Nelson and Woodard 1953).

No significantly increased tumour incidences were observed in seven monkeys fed a low dose of Solvent Yellow 2 in the diet for 5 years (a total dose of 15.27 g/monkey, approximately 1.05 mg/kg-bw per day) and observed for another 15 years (Takayama et al. 2008), nor in hamsters fed Solvent Yellow 2 in the diet for a short period of time (42 weeks, total dose of 1155 mg/hamster, equivalent to 28 mg/kg-bw per day) (Fischer 1954; Terracini and Della Porta 1961; IARC 1975f) or in guinea pigs fed 600–8000 mg/kg of Solvent Yellow 2 in the diet (24–320 mg/kg-bw per day) for 18 months (Orr 1940; IARC 1975f).

Skin tumours were observed in all six rats that were applied 1 mL of a 0.2% solution of Solvent Yellow 2 (in acetone) on the skin twice weekly for 90 weeks. No dye stained liver or liver lesions were observed (Fare 1966; IARC 1975f). However, skin or liver tumours were not observed in 40 mice that were skin painted with a 1% or 3% solution of Solvent Yellow 2 (in benzene) twice per week for 1 year (Roussy and Guérin 1946; IARC 1975f).

In addition, liver tumours were observed in rats following intraperitoneal injection (Mori and Nakahara 1940; Mori et al. 1956) or after implantation with Solvent Yellow 2 in the livers of male rats (Aterman 1987). Significantly increased liver tumour incidences were observed in newborn male B6C3F1 mice (female mice were much less susceptible) after receiving either a single intraperitoneal injection of Solvent Yellow 2 on postnatal day 12 or repeated intraperitoneal injections on postnatal days 1, 8, 15 and 22; such effects were not observed in newborn F344 rats received similar treatments (Delclos et al. 1984). Nevertheless, a same type of DNA adducts was detected in the livers of both treated rats and mice (Delclos et al. 1984). Following subcutaneous injection of Solvent Yellow 2, liver tumours and/or local tumours were observed in some mouse strains (C57BL, CBA or mixed stock) (Law 1941; Kirby 1945a, b), but not in others (DBA, A or C) (Law 1941; Andervont and Edwards 1943a) or in rats (strain CD and a non-specified strain) (Maruya and Tanaka 1936; Poirier et al. 1967). Newborn Swiss mice received subcutaneous injection of Solvent Yellow 2 on each of the first 5 days of life developed liver tumours at 1 year of age (Roe et al. 1971), whereas newborn Wistar rats received a single subcutaneous injection of Solvent Yellow 2 within the first 12 hours of life did not develop tumours at 380 days of age (Baba and Takayama 1961).

The genotoxic potential of Solvent Yellow 2 has been investigated *in vivo* and *in vitro*. The clastogenicity of Solvent Yellow 2 has been observed in several *in vivo* tests. Significantly increased micronuclei were observed in the liver, peripheral blood and bone marrow (Suzuki et al. 2005, 2006, 2009; Takasawa et al. 2010) of orally dosed rats, in the bone marrow of orally dosed mice and in the blood of mice following intraperitoneal injection of Solvent Yellow 2 (Salamone et al. 1981; Biswas and Khuda-

Bukhsh, 2005), although negative micronuclei test results in the blood samples were also observed in orally dosed mice (Morita et al. 1997a, b) or after intraperitoneal injection (Kirkhart 1981; Tsuchimoto and Matter 1981). Significantly increased chromosomal aberrations following oral administration (Biswas and Khuda-Bukhsh 2005) and significantly increased sister chromatid exchange following intraperitoneal injection (Parodi et al. 1983; Tucker et al. 1993) were observed in mouse bone marrow (Morita et al. 1997a). Solvent Yellow 2 produced DNA damages (comet assay) in the stomach, colon, urinary bladder, lung, liver and bone marrow of mice following oral administration or intraperitoneal injection and in the mouse kidney via intraperitoneal injection (Tsuda et al. 2000, 2001; Sekihashi et al. 2001). Solvent Yellow 2 also caused mitotic index changes in sperms of orally dosed mice (Biswas and Khuda-Bukhsh 2005) and morphological changes in the sperms of the treated mice following intraperitoneal injection (Wyrobek et al. 1981, 1983); however, the latter effect was not observed in another study using a higher dose level (Topham 1980; Wyrobek et al. 1983). In addition, in cancer studies in newborn rats and mice (Delclos et al. 1984), three types of DNA adducts, i.e., *N*-(deoxyguanosin-8-yl)-4-aminoazobenzene, *N*-(deoxyguanosin-8-yl)-*N*-methyl-4-aminoazobenzene and 3-(deoxyguanosin-N^2-yl)-)-*N*-methyl-4-aminoazobenzene, were detected in the livers of treated F344 rats and B6C3F1 mice 24 hours after a single intraperitoneal injection of Solvent Yellow 2 at 12 days of age (Delclos et al. 1984). In *Drosophila*, Solvent Yellow 2 induced mitotic chromosomal recombination in somatic cells in the eye mosaic assay (Vogel and Nivard 1993), but did not induce gene mutations in the sex-linked recessive lethal assay (Demerec 1949).

In vitro, Solvent Yellow 2 induced gene mutations in mammalian cells and in bacteria, for which metabolic activation was essential. Positive gene mutation tests results were observed in mouse lymphoma cells with S9 prepared from Aroclor-induced rat liver (Mitchell et al. 1988; Myhr and Caspary 1988), while negative results were observed in V79 cells with a different S9 preparation (Fassina et al. 1990). In Ames tests, although the results from different studies were not very consistent and Solvent Yellow 2 precipitation was observed in some of these tests (Suzuki et al. 2006), positive results in *S. typhimurium* strains TA98 and TA100 were repeatedly observed in different tests with metabolic activation; positive results in strain TA1538 with induced mouse S9 were reported in one of the studies, and negative results were reported in strains TA1535, TA1537 and TA97 and in *E. coli* WP2 *uvrA*$^-$, with and without S9 (Commoner et al. 1974; Dunkel et al. 1984; Zeiger et al. 1987; Hakura et al. 2005; Suzuki et al. 2006). Solvent Yellow 2 caused chromosomal damages in mammalian cells in chromosomal aberration assays with and without (weak positive) metabolic activation (Suzuki et al. 2006) and in sister chromatid exchange assays with metabolic activation (Perry and Thomson 1981; Tucker et al. 1993). Solvent Yellow 2 also caused unscheduled DNA synthesis in human HeLa cells with metabolic activation (Martin et al. 1978) and in rat hepatocytes (Mori et al. 1986). It did not induce DNA damage/repair response in *E. coli* (with and without metabolic activation) or in *B. subtilis* (no metabolic activation) (Kada et al. 1972; Rosenkranz and Poirier 1979; Leifer et al. 1981). Solvent Yellow 2 did not induce mitotic chromosome recombination in yeast with metabolic activation (Simmon 1979b; Zimmermann et al. 1984).

In addition, Solvent Yellow 2 induced Rauscher leukemia virus–infected rat embryo cell transformation, but not mouse BALB/c3T3 cell transformation, whereas the cell transformation results in the Syrian hamster embryo cell line were questionable (Freeman et al. 1973; Dunkel et al. 1981; Heidelberger et al. 1983). It has shown that intraperitoneal injection of Solvent Yellow 2 in pregnant hamsters increased the transformation ability of embryonic cells *in vitro* (DiPaolo et al. 1973).

7.2.1.3.3 Other health effects

Several studies reported other health effects of Solvent Yellow 2 in orally dosed non-human primates and rodents. Liver cytotoxicity, including cell damage and inflammatory response (Kupffer cell proliferation and enlargement and macrophage infiltration), was observed in CF1 mice fed 0.5% Solvent Yellow 2 in the diet (650 mg/kg-bw per day) for 14 months, along with liver tumour development (Caballero et al. 2004). When Common Cotton-eared Marmosets (New World monkey) were given 2% Solvent Yellow 2 solution by gavage (56 mg/kg-bw per day) for 5, 10 or 15 days, depressed body weights, decreased red and white blood cell counts, decreased hemoglobin levels as well as other altered blood biochemical parameters were observed, indicating anemia and liver injury. In addition, thyroid C-cell hyperplasia, leukocyte aggregation in the liver, tubular dilatation in the kidney, cell infiltration in the thyroid, kidney and adrenal gland as well as significantly increased relative liver, kidney, spleen and adrenal weights were observed; 2 of 15 treated monkeys died on day 10 (Matsumoto et al. 1986). Matsumoto et al. (1986) also reported similar toxicological findings in treated rats. Acute toxicity, including cyanosis and, occasionally, exertional dyspnea, was observed in female Sprague-Dawley (SD) rats following a single intraperitoneal injection of Solvent Yellow 2 (Lin and Wu 1974). Methemoglobin formation was observed in male SD rats 1–10 hours following an intraperitoneal injection of Solvent Yellow 2 (Lin and Wu 1973).

Solvent Yellow 2 was classified by the European Commission as a Category 1 skin sensitizer – “May cause an allergic skin reaction” (European Commission 2008). In a skin patch test, 31 of 6203 human patients showed positive sensitization reactions to Solvent Yellow 2 (Seidenari et al. 1997).

7.2.1.3.4 Summary

Overall, Solvent Yellow 2 exhibited carcinogenicity and genotoxicity and elicited haematological effects in experimental animals. It induced gene mutations in some mammalian cells and bacterial strains *in vitro*. It showed clastogenicity and caused DNA damages *in vivo* and *in vitro*. It produced DNA adducts *in vivo*. It also induced cell transformation of some mammalian cells. Solvent Yellow 2 induced liver tumours in rats and liver nodules/tumours in mice via oral administration. It also induced bladder papillomas in dogs via oral administration and skin tumours in rats following dermal application. Evidence from *in vivo* and *in vitro* genotoxicity assays indicates a potent genotoxicity of Solvent Yellow 2, which may have played a role in Solvent Yellow 2-induced tumour formation. In addition, liver cytotoxicity was observed along with liver tumour formation in orally dosed mice, which may also have contributed to Solvent

Yellow 2–induced tumour formation. The methemoglobin effects elicited by Solvent Yellow 2 are likely mediated by the *N*-hydroxylation of its aryl amine metabolites.

7.2.1.4 Solvent Orange 3 (CAS RN 495-54-5)

In the toxicological studies identified, it is often unclear whether Solvent Orange 3 or its hydrochloride salt (also known as Basic Orange 2 or Chrysoidine, CAS RN 532-82-1) was tested. Basic Orange 2 is a water soluble substance with an experimental water solubility value of 20 g/L (Environment Canada and Health Canada 2014a). While no experimental water solubility value for Solvent Orange 3 is available, computer modelling predicts its water solubility to be 213.4 mg/L (Appendix B, Table B-4). It is expected that once dissolved, both Solvent Orange 3 and Basic Orange 2 will release the same aromatic azo moiety, i.e., 2,4-diaminoazobenzene. Therefore, the two substances are considered to be toxicologically equivalent, although under different testing conditions, their relative absorption and subsequent target organ concentrations may differ.

Both Solvent Orange 3 and Basic Orange 2 were classified by the European Commission as Category 2 mutagens – “Suspected of causing genetic defects” (European Commission 2008). Basic Orange 2 was classified by IARC as a Group 3 substance – “Not classifiable as to its carcinogenicity to humans” (IARC 1987a).

The health effects of Basic Orange 2 have been reviewed by IARC (1975d; 1987b) and were also assessed along with Certain Azo Basic Dyes (Environment Canada and Health Canada 2014a). Available toxicological data related to chrysoidine have been presented in the assessment for Certain Azo Basic Dyes. These data are considered applicable to Solvent Orange 3; a summary of these health effects is presented below.

In a cancer bioassay for Basic Orange 2 that has been reviewed by IARC, liver cell tumours, leukaemia and reticulum cell sarcomas were observed in mice fed a low vitamin diet containing Basic Orange 2 (260 mg/kg-bw per day) for 13 months (IARC 1975d; 1987b). In genotoxicity tests (with Solvent Yellow 3 or Basic Orange 2), positive results were observed in bacterial reverse gene mutation tests (Ames) in *S. typhimurium* strains TA98, TA100, TA1537 and TA1358, but not in strain TA1535, with metabolic activation; without metabolic activation, the Ames results were all negative. Positive results in DNA damage/repair assays, measured as unscheduled DNA synthesis, were observed in rat livers following oral administration and *in vitro* in rat hepatocytes. Negative results were observed in a micronucleus test in mice and in gene mutation tests (sex-linked recessive lethal test) in *Drosophila*. Under reductive Ames test conditions (with flavin mononucleotide [FMN]), the gene mutation frequency in *S. typhimurium* strain TA100 was dramatically reduced, indicating that azo bond cleavage reduced the mutagenicity of 2, 4-diamino-azobenzene moiety in this bacterial strain.

Limited data regarding other health effects have been identified. Haematological effects were observed in rats that were given chrysoidine (total 670 mg/rat) in drinking water for 21 days.

7.2.1.5 Solvent Yellow 3 (CAS RN 97-56-3)

7.2.1.5.1 Absorption, distribution, metabolism and excretion

Following oral administration (feeding), the radioactivity of ^{3}H-labelled Solvent Yellow 3 was detected in the liver of C57 or CBA mice (Müller 1967; Lawson 1970). When male and female Donryu rats were injected with Solvent Yellow 3 into the stomach through a catheter, hydroxyl- and hydroxymethyl- derivatives of Solvent Yellow 3 and their corresponding conjugates with glucuronic or sulfuric acid as well as the *N*-glucuronide of Solvent Yellow 3 were detected in the bile (Samejima et al. 1967). In rats dermally applied Solvent Yellow 3 (3.5 mg, every other day for 3 months), increased anaerobic glycolysis in the liver and kidney, increased cytochrome c oxidase in the kidney and stimulated oxygen consumption were observed (Arzamastsev 1970). *In vitro* incubating of Solvent Yellow 3 with rat liver microsome and nicotinamide adenine dinucleotide phosphate (NADPH) and nicotinamide adenine dinucleotide (NADH) produced *N*-hydroxy- and 4′-hydroxy-Solvent Yellow 3 and a smaller amount of 2′-hydroxymethyl-3-methyl-4-aminoazobenzene (Degawa et al. 1982). The azo linkage of Solvent Yellow 3 was reported to be easily reduced by yeast to generate *o*-toluidine and 2-methyl-1,4-phenylenediamine, and the latter was also detected in dosed rabbits (IARC 1975b). However, the azo linkage of Solvent Yellow 3 was not reduced by the skin bacteria *Staphylococcus epidermidis* and *Micrococcus luteus* under aerobic conditions *in vitro* (BRI 2012).

7.2.1.5.2 Carcinogenicity and genotoxicity

Solvent Yellow 3 has been classified by IARC as a Group 2B substance – “Possibly carcinogenic to humans” (IARC 1987a), by the European Commission as a Category 1B carcinogen – “May cause cancer” (European Commission 2008) and by the US NTP as “Reasonably anticipated to be a human carcinogen” (NTP 2011). Solvent Yellow 3 is one of the EU22 aromatic amines.

The carcinogenic potential of Solvent Yellow 3 through oral intake has been investigated in mice, rats, hamsters and dogs. Increased incidences of liver tumours were consistently reported in these studies. Liver tumours generally appeared within a year of exposure, varying by the dose levels and animal species and strains used in the studies. In mice, increased liver tumour incidences were prominent in the treated females (Andervont 1944; Silverstone 1948; Shelton 1955; Waterman and Lignac 1958; Müller 1967; Walker et al. 1972; IARC 1975b; Pritchard and Butler 1984; Sax 1986; Popova 1989). Significant liver tumour induction was observed in female DBA mice at 45 weeks of age after receiving 31 mg/kg-bw per day (time-adjusted dose level) of Solvent Yellow 3 in the diet for 23 weeks, compared with the untreated C3H male mice (Silverstone 1948), and in female CF1 mice fed 600 mg/kg of Solvent Yellow 3 in the diet (78 mg/kg-bw per day) for 6 months, compared with the untreated female mice (Walker et al. 1972). Liver tumours were observed in both sexes of strain C mice (which also developed pulmonary and lung hemangioendotheliomas) after receiving approximately 15–33 mg/kg-bw per day of Solvent Yellow 3 in the diet for 223 days (Andervont 1944) and in heterogeneous type B mice fed 67 mg/kg-bw per day of

Solvent Yellow 3 in the diet for 400 days (Waterman and Lignac 1958). In addition, liver tumour incidences were significantly increased in the offspring (both sexes of F_1 and female F_2) of CBA female mice received 130 mg/kg-bw per day of Solvent Yellow 3 orally on gestation days 17–19. The liver tumour incidences were also significantly increased in the F_0 females; but they were reduced in F_2 and F_3 males (Popova 1989). In rats, it was reported in several studies that Solvent Yellow 3 induced liver tumours at 200 mg/kg (10 mg/kg-bw per day) and above dose levels. These studies were published in the 1930s and have been reviewed by IARC (1975b). It was reported in a study, which has not been reviewed by IARC (1975b), that significantly increased liver tumour incidences were observed in both sexes of rats fed 0.1% Solvent Yellow 3 in the diet (50 mg/kg-bw per day) for 473 days (Waters 1937). Significantly increased incidences of liver tumours and urinary bladder tumours were observed in both sexes of hamsters fed 0.1% Solvent Yellow 3 in the diet (61.2 mg/kg-bw per day) for 49 weeks. In addition, mammary tumours in the treated female hamster and gall bladder tumours in both sexes of the treated animals were also observed (Tomatis et al. 1961). Similarly, Solvent Yellow 3–induced liver, urinary and gall bladder tumours were also observed in dogs fed 5 mg/kg-bw per day of Solvent Yellow 3 in the diet for 30–62 months; although no control dogs were used in the study, such tumours were not observed in dogs that were treated with other chemicals in the same laboratory (Nelson and Woodard 1953).

Following dermal exposure, Solvent Yellow 3-induced tumours were reported in the studies reviewed by IARC (1975b). Solvent Yellow 3 induced liver tumours in mice that received skin painting of 0.5% or 1% Solvent Yellow 3 in benzene solution every other day for 6 months (Morosenskaya 1938, 1939) or 1% Solvent Yellow 3 three times per week for 8 months (Khramkova and Guelstein 1965). When pregnant C3H/A mice received skin painting of Solvent Yellow 3 during pregnancy and lactation, 30% of the F_1 offspring developed lung adenomas and 65% had liver tumours. When female mice were treated with Solvent Yellow 3 dermally only during pregnancy, 19% of the F_1 offspring had lung tumours and 43% had liver tumours. In the control mice, the incidences of liver tumours and lung adenomas were 2% and 5%, respectively (Gelstein 1961). However, benzene used in these studies may be a confounding factor as dermal application of benzene has induced papillomas, spindle cell tumors and granulocytic leukemia in transgenic mice carrying the v-*Ha-ras* oncogene, which increases their susceptibility to carcinogens (Spalding et al, 1999; Fremch and Saulnier 2000l; NTP 2011).

A number of studies have focused on the differential susceptibility of mouse strains to the Solvent Yellow 3–induced liver tumour formation. These studies were mainly conducted via subcutaneous injection (Law 1941; Andervont 1942, 1950, 1958; Andervont and Edwards 1943b; Nishizuka et al. 1965; IARC 1975b; Kaledin et al. 1978) and some via intraperitoneal injection (Akamatsu et al. 1967; Clayson et al. 1968). Solvent Yellow 3 exposure was associated with high incidences of liver tumours in some mouse strains, including A/Sn, CBA, SWR, DBA/2, A/He, C57BL, GR and DD, but had little effect in other strains, such as AKR, CC57BR and BALB (Timofeeva et al. 2008; Pakharukova 2011; Smetanina et al. 2011). In addition, newborn mice are more susceptible than adults and adult female mice are more susceptible than adult males; in

contrast, newborn male mice seem more susceptible than the newborn females (Nishizuka et al. 1965). Pulmonary tumours and hemangioendotheliomas were also observed in mice following subcutaneous injection of Solvent Yellow 3 (Andervont and Edwards 1943a; Andervont 1950; Nishizuka et al. 1965; IARC 1975b). A single subcutaneous injection of 0.4 or 0.7 mg of Solvent Yellow 3 in A/Jax mice on postnatal day 1 induced liver and pulmonary tumours in both sexes at 15 months of age (Nishizuka et al. 1965). Liver tumours were also observed in rats and fowl after receiving Solvent Yellow 3 via subcutaneous injection (IARC 1975b).

In addition, bladder papillomas were observed in rabbits that were either daily instilled with 1% Solvent Yellow 3 into the bladder for 357 days or subcutaneously injected with 1% Solvent Yellow 3 for 216 days; however, no control data were available in the report (IARC 1975b). Significantly increased incidences of bladder carcinomas were observed in mice after implantation of paraffin wax pellets containing 12.5% Solvent Yellow 3 into the bladders for 40 weeks (Clayson et al. 1968). Interscapular tumours in the brown adipose tissue were observed in 81% of the treated mice that were subcutaneously implanted 10 mg of Solvent Yellow 3 at monthly intervals for 8 months (Sax 1986).

The genotoxic potential of Solvent Yellow 3 has been investigated in a wide range of *in vivo* and *in vitro* bioassays. Gene mutations were detected in the liver, colon, kidney and bladder tissues of Muta^{TM} Mouse following intraperitoneal injection of Solvent Yellow 3 (Ohsawa et al. 2000; Kohara et al. 2001). Significantly increased chromosomal damages, measured by micronucleus assay (Ohsawa et al. 2000; Kohara et al. 2001), chromosomal aberration assay (Kim 1973) and sister chromatid exchange assay (Parodi et al. 1983), were observed in mice and/or rats after intraperitoneal or subcutaneous injection of Solvent Yellow 3. Solvent Yellow 3 induced DNA damage/repair, measured by unscheduled DNA synthesis tests, in the hepatocytes of orally dosed rats and mice (Kornbrust and Barfknecht 1985a, b; Barfknecht et al. 1987; Lake et al. 2001). Solvent Yellow 3-produced DNA damages were also detected by comet assay in the liver, colon and lung of orally dosed mice, in the lung, stomach, colon, bladder and brain of orally dosed rats and in the mouse stomach, colon, liver, bladder, lung and brain following intraperitoneal injection (Sasaki et al. 2000; Tsuda et al. 2000; Sekihashi et al. 2002); negative results were observed in the alkaline elution assay (DNA damage) in rats following intraperitoneal injection (Parodi et al. 1981). Solvent Yellow 3 did not induce gene mutations in *Drosophila* (Edwards and Combes 1981).

In vitro, Solvent Yellow 3 exhibited mutagenicity and some clastogenicity. It also caused DNA damages. Solvent Yellow 3 induced gene mutations in bacteria with metabolic activation. Positive Ames test results in *S. typhimurium* strains TA98, TA100 and TA1538, but not in strains TA1535 and TA1536, and mixed results in strain TA1537 were observed (Ames et al. 1973; McCann et al. 1975; Yahagi et al. 1975; Rosenkranz and Poirier 1979; Simmon 1979b; Simmon et al. 1979; Kawachi et al. 1980a,b; Kawajiri et al. 1980; Muller et al. 1980; Arni and Mueller 1981; Parodi et al. 1981; Degawa et al. 1982; Longstaff et al. 1984; Mamber et al. 1984; Kawano et al. 1985; Miyagoshi et al. 1985; Cameron et al. 1987; Zeiger et al. 1992; Cheung et al. 1994; Lee et al. 1994;

Ohsawa et al. 2000; Hakura et al. 2005; Mikhailova et al. 2005). Solvent Yellow 3 also induced gene mutations in mouse lymphoma cells (Cameron et al. 1987), in *E. coli* (Scherr et al. 1954) and in mould *Neurospora crassa* (Brockman et al. 1984), but it did not induce gene recombination in yeast (Simmon 1979a; Zimmermann et al. 1984). Solvent Yellow 3 induced sister chromatid exchanges in rat tumour cells co-cultured with hamster cells (Abe and Sasaki 1982) and unscheduled DNA syntheses in primary rat and hamster hepatocytes (Kornbrust and Barfknecht 1984; Mori et al. 1986; Barfknecht et al. 1987; Kornbrust and Dietz 1987), but it did not induce chromosomal aberrations or sister chromatid exchanges in hamster cells (Abe and Sasaki 1977; Ishidate and Odashima 1977; Matsuoka et al. 1979; Kawachi et al. 1980a, b; Latt et al. 1981; Ohsawa et al. 2000). Solvent Yellow 3 induced DNA damage/repair in *S. typhimurium*, *E. coli* and *B. subtilis* in various tests, including *umu* test, SOS assay, *rec*-assay, *pol A*-assay and prophage induction test (Ichinotsubo et al. 1977; Rosenkranz and Poirier 1979; Kada et al. 1980; Kawachi et al. 1980a, b; Leifer et al. 1981; Suter and Jaeger 1982; Mamber et al. 1984, 1986; Nakamura et al. 1987; Yamazaki et al. 1992; Mersch-Sundermann et al. 1994; Oda et al. 1995; Reifferscheid and Heil 1996; Shimada et al. 1996).

In addition, Solvent Yellow 3 induced cell transformation in Syrian hamster embryo cells (Heidelberger et al. 1983).

7.2.1.5.3 Other health effects

Transplacental effects of Solvent Yellow 3 have been observed in pregnant C3H/A mice via dermal application (Gelstein 1961) and in pregnant CBA mice via oral administration (Popova 1989). Increased incidences of liver and/or lung tumours were observed in the offspring of the treated mice.

Solvent Yellow 3 was classified by the European Commission as a Category 1 skin sensitizer – “May cause an allergic skin reaction” (European Commission 2008). It caused skin sensitization in guinea pigs (Schäfer et al. 1978). Solvent Yellow 3 tested positive in patch tests in some human patients (Meara and Martin-Scott 1953; Zina and Bonu 1965; Castelain 1967; Jordan and Dahl 1972; Foussereau et al. 1973; Bedello et al. 1982; Foussereau 1985; Conde-Salazar et al. 1991; Bajaj et al. 2000).

7.2.1.5.4 Summary

Overall, Solvent Yellow 3 exhibited carcinogenicity and genotoxicity in experimental animals. It induced gene mutations *in vivo* and *in vitro*. It showed clastogenicity *in vivo* and to a lesser extent *in vitro*. It caused DNA damages *in vivo* and *in vitro*. Following oral administration, Solvent Yellow 3 induced liver tumours in mice, rats, hamsters and dogs, induced urinary bladder tumours and gall bladder tumours in hamsters and dogs and induced lung hemangioendotheliomas in mice. It also induced liver tumours in mice via dermal exposure. Evidence from a wide range of *in vivo* and *in vitro* genotoxicity assays indicates a potent genotoxicity of Solvent Yellow 3, which may have played a role in Solvent Yellow 3–induced tumor formation.

7.2.1.6 Solvent Yellow 77 (CAS RN 2832-40-8)

7.2.1.6.1 Absorption, distribution, metabolism and excretion

In vitro dermal absorption data for Solvent Yellow 77 were presented in the Exposure Assessment section (ETAD 1994, 1995, 1997); the dermal penetration rate of Solvent Yellow 77 through human skin was approximately 10 times slower than that through pig skin. Solvent Yellow 77 was degraded (about 9%) when incubating with *B. subtilis* laccase for 24 hours (Pereira et al. 2009).

7.2.1.6.2 Carcinogenicity and genotoxicity

Solvent Yellow 77 has been classified by the European Commission as a Category 2 carcinogen – “Suspected of causing cancer” (European Commission 2008) and by IARC as a Group 3 substance – “Not classifiable as to its carcinogenicity to humans” (IARC 1990).

The carcinogenic potential of Solvent Yellow 77 has been investigated in dietary studies in mice and rats (NTP 1982a). When B6C3F1 mice were fed 2500 or 5000 mg/kg of Solvent Yellow 77 (purity 87.6%) in the diet (325 or 650 mg/kg-bw per day) for 103 weeks, significantly increased incidences of hepatocellular adenomas were observed in the treated females at both dose levels. The incidences of hepatocellular carcinomas were also increased in the treated females, but the results were not statistically significant. In addition, significantly increased incidences of alveolar/bronchiolar adenomas in the treated males at the high dose level and malignant lymphomas in the treated females in a dose-related manner were observed; however, the authors considered these not to be unequivocal evidence of the treatment related effects. When Fischer 344/N rats were fed 5000 or 10000 mg/kg of Solvent Yellow 77 in the diet (250 or 500 mg/kg-bw per day) for 103 weeks, significantly increased incidences of neoplastic nodules in the livers were observed in the treated males at both dose levels. Stomach tumours were observed in the treated males, but the results were not statistically significant. NTP (1982a) considered Solvent Yellow 77 to be carcinogenic to male, but not female, F344 rats and to be carcinogenic to female, but not male, B6C3F1 mice. Based on the results of this report, a lowest lower confidence limit on the benchmark dose for a 10% response ($BMDL_{10}$) value of 51.29 mg/kg-bw per day was

derived (Appendix H). In addition, the potential of Solvent Yellow 77 to induce bladder tumours was investigated by implantation of this dye in the bladders of stock mice. One adenoma and six carcinomas (7/23) were observed 25 weeks after the mice received cholesterol pellets containing Solvent Yellow 77 (concentration unknown) (Boyland et al. 1964). The results were considered to be of borderline significance by IARC (IARC 1975e).

The genotoxic potential of Solvent Yellow 77 has been investigated *in vivo* and *in vitro*. Solvent Yellow 77 caused DNA damages (comet assay) in the stomach, liver and brain of orally dosed ddY mice (Tsuda et al. 2000), but it did not cause DNA damages (alkaline elution on filter) in orally dosed SD rats (Kitchin and Brown 1994). Solvent Yellow 77 did not induce chromosomal damages, measured by micronuclei formation or chromosomal aberrations, in B6C3F1 mice following intraperitoneal injection (Shelby et al. 1993; Shelby and Witt 1995), but it induced chromosomal aberrations in frog larvae (Anderson 1996). Solvent Yellow 77 did not induce gene mutations in *Drosophila* in sex-linked recessive lethal tests (Foureman et al. 1994). Solvent Yellow 77 induced gene mutations *in vitro* in mouse lymphoma cells with and without (weakly positive) metabolic activation (Cameron et al. 1987; McGregor et al. 1988; NTP undated-b) and in bacteria with and without metabolic activation. Positive Ames test results were observed in *S. typhimurium* strains TA97, TA98, TA100, TA1537 and TA1538, but not in strain TA1535 (Cameron et al. 1987; IARC 1990; NTP 1983, 1993b; Zeiger et al. 1988), although one study reported negative results in strain TA100 without metabolic activation (Cameron et al. 1987). Under reductive Ames test conditions (with FMN), positive results were observed in *S. typhimurium* strains TA98 and TA100 (Cameron et al. 1987). Solvent Yellow 77 induced sister chromatid exchange in CHO cells without metabolic activation; the results were negative with metabolic activation (NTP 1985; Tennant et al. 1987a, b; Ivett et al. 1989). It did not induce chromosomal aberrations in CHO cells with and without metabolic activation (NTP 1985; Tennant et al. 1987a,b; IARC 1990). It induced DNA damage/repair in rat hepatocytes *in vitro* in unscheduled DNA synthesis assays (IARC 1990; Tennant et al. 1987a, b) and in bacterial cells, measured by *umu*/SOS response (Yasunaga et al. 2004).

In addition, Solvent Yellow 77 did not induce BALB/c-3T3 cell transformation without metabolic activation (Matthews et al. 1993).

7.2.1.6.3 Other health effects

In the NTP series tests in F344 rats and B6C3F1 mice (NTP 1982a), no animal deaths or signs of toxicity were observed in the treated animals in the single-day studies, up to dose level of 100000 mg/kg in the diet (approximately 5000 and 4000 mg/kg-bw for rats and mice, respectively). In the short-term (14 days) studies, body weight gain depression was observed in all treated animals. Splenic enlargement was noted in mice fed 25000 mg/kg or more of Solvent Yellow 77 in the diet (3250 mg/kg-bw per day), but not at 12500 mg/kg dose level (1625 mg/kg-bw per day, mouse oral short-term NOAEL). Depletion of fat deposits around the kidney, adrenal and heart and colour changes (dark red to black) in the spleen, kidney and liver were observed in the treated

rats, along with animal deaths (1/5 of each sex), at 50000 mg/kg (2500 mg/kg-bw per day) or higher dose levels, but not at 25000 mg/kg dose level (1250 mg/kg-bw per day, rat oral short-term NOAEL). In the subchronic (13 weeks) studies, proliferative lesions of the thyroid follicular cells as well as vacuolar degeneration of the pars distalis of the pituitary gland, hemosiderosis in the spleen and kidney pigmentation were observed in the treated rats at 5000 mg/kg (250 mg/kg-bw per day) and/or higher dose levels. Hemosiderosis of the renal tubular epithelium and spleen and centrilobular hepatocyte swelling were observed in the treated mice at 5000 mg/kg (650 mg/kg-bw per day) and above dose levels. In the chronic (2 years) studies, renal pigmentation in the treated female rats and focal hepatic cellular changes in the treated male rats at both dose levels (oral chronic LOEL = 250 mg/kg-bw per day) were observed; the nature of the pigment was not examined. Dose-related body weight gain reduction was observed in all treated mice and rats.

Solvent Yellow 77 was classified by the European Commission as a Category 1 skin sensitizer – “May cause an allergic skin reaction” (European Commission 2008). It caused allergic reaction in humans in skin tests (IARC 1990; Seidenari et al. 1997).

7.2.1.6.4 Summary

Overall, Solvent Yellow 77 exhibited carcinogenicity and genotoxicity and elicited haematological effects in experimental animals. It induced gene mutations *in vitro* in both bacteria and mammalian cells with and without metabolic activation. Solvent Yellow 77 caused DNA damages *in vivo* and *in vitro*. Solvent Yellow 77 induced liver nodules/tumours in female mice and male rats. It also induced hemosiderosis, likely via the *N*-hydroxylation of its aryl amine metabolites.

7.2.2 Sudan Dyes

Substances in the Sudan Dyes subset are similar to each other in terms of their structural characteristics, biological activities, potential activation pathways and modes of action. Solvent Orange 7 and Solvent Red 23 are also considered to belong to the Sudan Dyes subset however, the two substances were previously assessed and concluded under the Challenge Initiative of the CMP (Environment Canada and Health Canada 2009, 2011), and as significant new information related to their health assessments has not been identified, the previous conclusions on human health for these two substances has not been updated in the current assessment. Information on Solvent Orange 7 and Solvent Red 23 was however considered to support the read-cross approach for the other four Sudan Dyes being evaluated in the current assessment (Table 7-3).

In the sections below, the Sudan Dyes are characterized in terms of their structural similarity, common azo cleavage products, and potentially shared bioactivation pathways, followed by health effects assessments for the four individual Sudan Dyes being evaluated in the current assessment (Table 7-3). Data for Solvent Orange 7 and Solvent Red 23 are found in the previous Challenge assessments of these substances

(Environment Canada and Health Canada 2009, 2011). Data read-across among the substances in this health subset is considered an appropriate approach to characterize their human health effects.

Table 7-3. Sudan Dyes

Substance name (CAS RN)	Postulated azo bond reductive cleavage products (CAS RN)
Sudan I (842-07-9)	1-amino-2-naphthol (2834-92-6) Aniline (103-33-3)
Oil Orange SS (2646-17-5)	1-amino-2-naphthol (2834-92-6) *o*-Toluidine (95-53-4) [EU22 aromatic amine]
Solvent Red 1 (1229-55-6)	1-amino-2-naphthol (2834-92-6) *o*-Anisidine (90-04-0) [EU22 aromatic amine]
Sudan IV (85-83-6)	1-amino-2-naphthol (2834-92-6) *o*-Toluidine (95-53-4) [EU22 aromatic amine] Toluene-2,5-diamine (95-70-5) *o*-Aminoazotoluene (97-56-3)

Sudan Dyes all have a hydroxyl- group at the *ortho* position to the azo bond on their naphthalene rings, which enables a coplanar structure to be formed via hydrogen bonding. The coplanar structure, consisting of the benzene and naphthalene rings as well as a fused ring between the two former rings, may have influence on the bioactivities of these substances. In a SAR assay, 40 azo substances were tested for their ability to induce cytochrome P448[8]/P450 enzymes and associated monooxygenase activities, as well as uridine diphosphate-glucuronyltransferase (UDPGT) activity in rat liver following intraperitoneal injection. Only lipophilic azo dyes with 1-azo-2-naphthol or 1-azo-2-naphthylamine moieties, including the members of Sudan Dyes (except Solvent Red 1, which was not tested), induced these enzyme activities (Fujita et al. 1984). Other studies also reported similar bioactivities among Sudan Dyes. Increased CYP1A1 messenger RNA (mRNA) and protein levels or enzyme activities were detected in the livers of rats and mice that were treated with some Sudan Dyes, including Sudan I, Solvent Orange 7, Solvent Red 23 and Sudan IV, via intraperitoneal injection; Sudan I appeared to be the most potent inducer (Lubet et al. 1983; Refat et al. 2008). Induced NAD(P)H:quinone reductase was observed in the mouse liver cells that were treated with these Sudan Dyes *in vitro* (De Long et al. 1986, 1987).

Azo bond reductive cleavage is a common metabolic pathway for Sudan Dyes. Bacterial-mediated azo bond reduction was observed in Sudan I, Solvent Orange 7, Solvent Red 23 and Sudan IV and the azo bond reductive products of Sudan I generated *in vivo* or by skin tissue *in vitro* were also detected (details presented in the individual substance sections below). Azo bond reduction could potentially release 1-amino-2-naphthol from all the substances in this subset and various aromatic amines

[8] The nomenclature of P448 has been changed to P450 family 1 (Rodrigues et al. 1989); P448 enzymes will be referred to as P450 family 1 hereafter in the report.

from individual substances. These azo bond reductive products have exhibited some common health effects. 1-Amino-2-naphthol has shown severe cytotoxicity, as it bound to erythrocytes *in vivo* (Hart et al. 1986), and generated active oxygen species (Nakayama et al. 1983) and inhibited bacterial growth *in vitro* (Pan et al. 2012). Other aromatic amines, at least one from each parent substance, have shown some hematologic effects. Aniline has caused methemoglobinemia and subsequent hemosiderosis and spleen tumour formation in experimental animals (Health Canada 2011). Human exposure to *o*-toluidine was associated with methemoglobinemia, hemoglobin adduct formation and urinary bladder tumour formation. It also caused spleen fibromas and sarcomas, mesotheliomas of abdominal cavity or scrotum, hemangiomas and hemangiosarcomas, hepatocellular carcinomas and adenomas, transitional cell carcinomas of the urinary bladder, fibromas and fibrosarcomas of subcutaneous tissues, fibroadenomas or adenomas of mammary glands, and peritoneal sarcomas in experimental animals (IARC 2012). *o*-Anisidine induced methemoglobin formation, hemosiderosis, hyperemia, hematopoiesis in the spleen, and bladder and thyroid tumour formation in experimental animals (ECJRC 2002).

Additional metabolic activation pathways have been observed in Sudan I, which are mediated by cytochrome enzymes or peroxidases resulting in electrophilic and reactive intermediates, such as benzenediazonium ion (Stiborová et al. 2009). Benzenediazonium ion–DNA adducts have been detected in rat liver after intraperitoneal injection of Sudan I (Stiborová et al. 2006), which may account for the mutagenicity of Sudan I and subsequent liver neoplastic nodule formation in Sudan I–treated experimental rats (details presented in the Sudan I section below). Activation of Sudan I via these pathways appears to require the hydroxyl- substituent at the *ortho* position to the azo bond linkage to form an intramolecular hydrogen bond to stabilize the coplanar structure, which is common to the members of this health subset, suggesting that the Sudan I activation pathways may also be common for other Sudan Dyes.

7.2.2.1 Sudan I (CAS RN 842-07-9)

7.2.2.1.1 Absorption, distribution, metabolism and excretion

Following oral administration or intraperitoneal injection of Sudan I in albino rats, conjugates of 1-amino-2-naphthol and 1-phenylhydrazo-2-naphthol were detected in rat urine. Glucuronides of hydroxy-l-phenylazo-2-naphthol (4′-, 6- and 4′,6-) were detected in both bile and urine (Childs et al. 1967). Sudan I induced cytochrome P450 (mainly CYP1A1) and NAD(P)H:quinone enzyme expression in rats following intraperitoneal injection of Sudan I (Fujita et al. 1984; Refat et al. 2008; Stiborová et al. 2013). In orally dosed rabbits, the main metabolites of Sudan I were free or conjugated *p*-aminophenol (44%). Other metabolites, including aniline and its metabolites, 1-*p*-hydroxyphenylazo-2-naphthol and its conjugates and 1-amino-2-naphthol as well as unchanged Sudan I were also detected in the urine (Daniel 1962). In another oral study in rabbits, glucuronides of hydroxy-1-phenylazo-2-naphthol (4′-, 6- and 4′, 6-) were detected in both bile and urine of the treated rabbits, and *N*-glucuronides of 1-phenylhydrazo-2-

naphthol and 4′-hydroxy-l-phenylhydrazo-2-naphthol as well as 1-amino-2-naphthol conjugates were detected in the urine (Childs and Clayson 1966).

Available dermal absorption data as well as the skin-mediated azoreduction of Sudan I are presented in the Exposure Assessment section.

Sudan I was metabolized *in vitro* by rat, rabbit, minipig and human (recombinant) liver enzymes (mainly by CYP1A1, also by CYP3A), by horseradish peroxidase and by prostaglandin H synthesis (Semanská et al. 2008; Dračínský et al. 2009; Stiborová et al. 2009). Metabolic activation of Sudan I by cytochrome P450 enzymes or peroxidase leads to the formation of reactive metabolites, such as benzenediazonium. Benzenediazonium–formed DNA adducts, 8-(phenylazo)guanine, were detected in rat liver following intraperitoneal injection and *in vitro* with calf thymus DNA (Stiborová et al. 1995, 2006). In addition, Sudan I suppressed 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced chromosomal aberrations in rat bone marrow, suggesting that Sudan I may have induced metabolic enzymes that are related to the detoxification process of DMBA (Ito et al. 1982). *In vitro* exposure of human first-trimester placental explants to Sudan I resulted in increased estrogen hydroxylase and catechol-*O*-methyl transferase activities (Barnea and Avigdor 1990), as well as monooxygenase aryl hydrocarbon hydroxylase (Barnea and Avigdor 1991) and quinone reductase activities (Barnea et al. 1993). The azo linkage of Sudan I was reduced after incubating with intestinal contents, intestinal tissue or rat or human intestinal bacteria (Childs et al. 1967; Xu et al. 2007, 2010), as well as with the environmental bacterium *Shewanella oneidensis* MR-1 (Ji et al. 2012).

7.2.2.1.2 Carcinogenicity and genotoxicity

Sudan I has been classified by the European Commission as a Category 2 carcinogen – “Suspected of causing cancer” and a Category 2 mutagen – “Suspected of causing genetic defects” (European Commission 2008), and it was classified by IARC as a Group 3 substance – “Not classifiable as to its carcinogenicity to humans” (IARC 1987a).

The carcinogenic potential of Sudan I via oral administration has been investigated in rats and mice. When F344 rats were fed 250 or 500 mg/kg of Sudan I (purity 94.1%) in the diet (12.5 or 25 mg/kg-bw per day) for 103 weeks, significantly increased incidences of neoplastic nodules in the liver were observed in both sexes of the rats in the high-dose group, which was considered by the authors to be indicative of the carcinogenic potential of Sudan I (NTP 1982b). Based on the results of this study, a lowest $BMDL_{10}$ value of 5.54 mg/kg-bw per day was derived (Appendix H). No clear evidence of Sudan I–induced tumours was observed in B6C3F1 mice fed 500 or 1000 mg/kg of Sudan I in the diet (65 or 130 mg/kg-bw per day) for 103 weeks (NTP 1982b). In an old study with limited information, tumours were not observed in 17 of 20 rats fed 1000 mg/kg of Sudan I in the diet (50 mg/kg-bw per day) for at least 1 year (Hackmann 1951). Similarly, Sudan I did not induce tumours in CBA and stock mice fed 1000 mg/kg of Sudan I in the diet (130 mg/kg-bw per day) for 12 months (IARC 1975i). Sudan I

exhibited tumour promotion, but not tumour initiation, activity in orally dosed F344 rats (Pitot et al. 1989; Dragan et al. 1991).

In addition, hepatomas were observed in male stock mice following subcutaneous injection of Sudan I (250 mg/kg-bw per application) at 3-week intervals for 51–60 weeks (IARC 1975i). When paraffin wax pellets containing Sudan I were implanted into the urinary bladders of albino, (C57 × IF)F_1 or female B6AF1/J mice (approximately 12–72 mg/kg-bw), significantly increased incidences of bladder tumours (benign and carcinomas) were observed in all treated animals after 40 weeks (IARC 1975i; Jull 1979).

The genotoxic potential of Sudan I has been investigated in a wide range of *in vivo* and *in vitro* studies conducted after the IARC (1975i) assessment was published. These studies provided some evidence of Sudan I-caused chromosomal and DNA damages *in vivo* and *in vitro*, DNA adducts *in vivo* and *in vitro* and gene mutations *in vitro* in mammalian cells and in bacteria. Sudan I–induced micronuclei were detected in the bone marrow of orally dosed AP_fSG, PVC and F344 rats and ICR (repeated dosing was required) and C57BL/$6J_f$BL10/Alpk (weakly positive), but not CRH, mice (Elliott et al. 1997; Gatehouse et al. 1991; Westmoreland and Gatehouse 1991; Kondo and Miyajima 1997; Wakata et al. 1998). Sudan I–induced micronuclei were also detected in the peripheral blood reticulocytes of orally dosed F344 rats and ICR mice (Kondo and Miyajima 1997; Wakata et al. 1998). Following intraperitoneal injection of Sudan I, positive results in sister chromatid exchange assay, equivocal results in micronuclei assay and negative results in chromosomal aberration assay were observed in B6C3F1 mice (NTP undated-c, d, e). Sudan I caused DNA strand breaks (comet assay) in the stomach and colon of orally dosed ddY mice and in the stomach, colon, lung and kidney of orally dosed Wistar rats (Tsuda et al. 2000; Sekihashi et al. 2002). Sudan I did not induce DNA strand breaks in the livers of orally dosed SD rats (Kitchin et al. 1992, 1994; Kitchin and Brown 1994). Sudan I did not exhibit a significant capacity to induce DNA repair in hetapocytes, measured by unscheduled DNA synthesis, in ApfSD, F344, PVGC and SD rats following oral administration (Kornbrust and Barfknecht 1985a, b; Mirsalis et al. 1985, 1989; Barfknecht et al. 1987; Westmoreland and Gatehouse 1991; Elliott et al. 1997). Sudan I produced DNA adducts in the livers of Wistar rats following intraperitoneal injection; 8-(phenylazo)deoxyguanosine was the major form (Stiborová et al. 2006). Sudan I did not induce gene mutations in *Drosophila* in the sex-linked recessive lethal assay (Foureman et al. 1994).

Sudan I induced gene mutations *in vitro* at the *hprt* locus in human B lymphoblastoid immortal cell lines, and the dose–response relationship of its mutagenicity was correlated with the expression levels of human cytochrome P450 enzymes in these cells (Johnson et al. 2010). In contrast, the gene mutation results in mouse lymphoma assays were mixed; in general, metabolic activation was required to achieve positive results (Cameron et al. 1987; McGregor et al. 1991). In bacteria, positive Ames test results have been observed in *S. typhimurium* strains TA97, TA98 and TA1538, but not in strains TA100, TA1535 and TA1537, with metabolic activation; mouse S9 showed a stronger capacity to activate the mutagenicity of Sudan I (Cameron et al. 1987; Zeiger

1987; NTP 1988b; Zeiger et al. 1988). A study abstract reported that Sudan I induced gene mutations in Ames tests with mouse S9, but not with rat S9 (Busk and Albanus 1978). Other studies also reported negative Ames test results in strain TA98 with rat or hamster S9 (Brown et al. 1978; Cameron et al. 1987). Under reductive Ames test conditions (with FMN or sodium dithionite), Sudan I did not induce gene mutations in strains TA98, TA100, TA1535, TA1537 and TA1538 (Brown et al. 1978; Cameron et al. 1987). In addition, Sudan I–induced gene mutations were observed in a photobacterium (Elmore and Fitzgerald 1990). Sudan I induced micronuclei and DNA strand breaks in human hepatoma (HepG2) cells (An et al. 2007; Zhang et al. 2008) and human B lymphoblastoid immortal cell lines (Johnson et al. 2010). It also induced sister chromatid exchanges, but not chromosomal aberrations, in CHO cells (Ivett et al. 1989). Sudan I metabolites, generated by liver microsomal enzymes, horseradish peroxidase or prostaglandin H synthase, formed DNA, RNA and protein adducts *in vitro* (Stiborová et al. 1999, 2009; An et al. 2007; Zhang et al. 2008). Sudan I did not induce unscheduled DNA synthesis in primary rat hepatocytes (Kornbrust and Barfknecht 1985a; Barfknecht et al. 1987).

In an attempt to investigate Sudan I–enhanced hepatocyte proliferation, equivocal results were observed in orally dosed F344 rats (Mirsalis et al. 1985, 1989). However, Sudan I significantly stimulated liver regeneration in rats that were partially hepatectomized followed by receiving 0.05% or more Sudan I in the diet for 10 days (Gershbein 1982). Sudan I induced BALB/c-3T3 cell transformation (Matthews et al. 1993) and enhanced human tumour cell proliferation *in vitro* (Ji et al. 2006a, b). Sudan I inhibited intercellular gap junction communication in Chinese hamster V79 cells (Tsuchiya et al. 1995).

7.2.2.1.3 Other health effects

In the NTP series dietary studies in F344 rats and B6C3F1 mice (NTP 1982b), chronic (2-year exposure) non-neoplastic effects, such as multifocal fibrosis of cardiac valve in both treated male and female rats, lung lymphoid hyperplasia in the treated male rats, and bile duct focal hyperplasia, atrophy of the pancreatic acinus and nephropathy in the treated female rats, were observed at 250 mg/kg (12.5 mg/kg-bw per day, the chronic oral exposure LOAEL) and above dose levels; no treatment-related effects were observed in the treated B6C3F1 mice in this study.

In the subchronic (90 days) study in rats, pigmentation in the tubular epithelium of kidney cortex was observed at 500 mg/kg (25 mg/kg-bw per day) dose level in all treated females; at higher dose levels, kidney pigmentation and hepatic degeneration in both sexes of the treated animals were observed. In mice, hepatic and renal hemosiderosis and splenic congestion were observed at 1000 mg/kg (130 mg/kg-bw per day) dose level in all treated animals; at higher dose levels, renal lesions and thymus lymphoid depletion in the treated males and animal deaths in both sexes were observed. In the short-term (2 weeks) studies, dark red intestines and congested livers were observed in the treated rats and mice at 6000 mg/kg dose level (300 mg/kg-bw per day for rats and 789 mg/kg-bw per day for mice), along with animal deaths. In the

single-day studies, no animal death or sign of toxicity was observed up to 100 000 mg/kg dose level (5000 mg/kg-bw per day for rats and 13 000 mg/kg-bw per day for mice). In another short-term study, rats were given 0.05% Sudan I in the diet for 10 days (25 mg/kg-bw per day); significantly increased relative liver weights were observed, but no other potential health effects were examined (Gershbein 1982).

Sudan I was classified by the European Commission as a Category 1 skin sensitizer – "May cause an allergic skin reaction" (European Commission 2008). Skin sensitization induced by Sudan I exposure has been reported in experimental animals and human cases (Kozuka et al. 1979; Kozuka et al. 1980; Sato et al. 1981; Fujimoto et al. 1985; Goh and Kozuka 1986; Imokawa et al. 1992; Ikarashi et al. 1996; Hariya et al. 1999).

7.2.2.1.4 Summary

Overall, under the conditions of the NTP cancer bioassays, Sudan I was carcinogenic in rats but not in mice of either sex. Sudan I exhibited some genotoxicity and induced hematological effects in experimental animals. It caused chromosomal and DNA damages *in vivo* and *in vitro* and induced gene mutations in mammalian cells and in bacteria *in vitro*. It induced neoplastic changes in rat liver via oral administration. It exhibited tumour promotion activity and induced mammalian cell transformation. Reactive metabolites of Sudan I bound to DNA and formed DNA adducts in rat liver, which could lead to gene mutation and subsequent tumour formation. In addition, a Sudan I metabolite, 1-amino-2-naphthol, has shown potent cytotoxicity, which could also contribute to the Sudan I–induced tumour formation.

7.2.2.2 Oil Orange SS (CAS RN 2646-17-5)

7.2.2.2.1 Absorption, distribution, metabolism and excretion

Oil Orange SS significantly induced cytochrome P450 family 1 enzymes and UDPGT activity in the liver of the treated rats following intraperitoneal injection (Fujita et al. 1982, 1984). The azo linkage of Oil Orange SS was not reduced by the skin bacteria *S. epidermidis* and *M. luteus* under aerobic conditions *in vitro* (BRI 2012).

7.2.2.2.2 Carcinogenicity and genotoxicity

Oil Orange SS has been classified by IARC as a Group 2B substance – "Possibly carcinogenic to humans" (IARC 1987a) and by the European Commission as a Category 2 carcinogen – "Suspected of causing cancer" and a Category 2 mutagen – "Suspected of causing genetic defects" (European Commission 2008).

The carcinogenic potential of Oil Orange SS via oral administration has been investigated in rats and mice. These studies were reviewed by IARC (1975g). Significantly increased incidences of intestinal tumours, mainly at the ileo-cecal junction, were observed in all 10 treated male albino mice (9 developed benign tumours, and 1 developed a carcinoma) that were fed 0.1% Oil Orange SS in the diet (66.7 mg/kg-bw per day) for 52 weeks and observed for 90 weeks (Bonser et al. 1956). No significant

tumour induction was observed in rats fed 0.03% Oil Orange SS in the diet (15 mg/kg-bw per day) for 44 weeks (only 1/20 rats developed ovarian fibroma and cystadenoma); higher doses caused animal deaths before 20 weeks (Allmark et al. 1956). A study abstract reported that no tumours were observed in rats fed 0.1% or 0.25% Oil Orange SS in the diet (50 or 125 mg/kg-bw per day) for 2 years or in dogs fed 0.01–0.2% Oil Orange SS in the diet (5–100 mg/kg-bw per day) for 26 days to 2 years (Fitzhugh et al. 1956). Following subcutaneous injection, Oil Orange SS induced intestinal tumours, in addition to spindle cell sarcomas at the injection sites, in albino mice (Bonser et al. 1954, 1956). Subcutaneous injection of Oil Orange SS did not induce tumours in Osborne-Mendel rats (Nelson and Davidow 1957). In addition, implantation of cholesterol pellets containing 12.5% Oil Orange SS into the urinary bladders of albino mice for 40 weeks induced benign tumours and carcinomas in the bladder (Clayson et al. 1958).

The genotoxic potential of Oil Orange SS has been investigated in some experiments *in vivo* and *in vitro*. Oil Orange SS induced chromosomal damage (micronucleus assay) in the bone marrow and peripheral blood of orally dosed SD rats (Wakata et al. 1998; Watanabe et al. 1999) and in the peripheral blood of CD-1 and MS/Ae mice following intraperitoneal injection (Morita et al. 1997). Oil Orange SS did not induce DNA damages (comet assay) in orally administered ddY mice (Tsuda et al. 2000). Oil Orange SS induced gene mutations in bacteria with metabolic activation. A study abstract reported positive Ames test results with mouse S9, but not with rat S9 (Busk and Albanus.1978). With rat or hamster S9, weakly positive or negative Ames test results in *S. typhimurium* strain TA100 and negative results in strain TA98 were reported (Miyagoshi et al. 1983; Zeiger et al. 1992). Oil Orange SS did not induce DNA damage/repair (rec assay) in *B. subtilis* (Kada et al. 1972).

Oil Orange SS significantly stimulated liver regeneration in rats that were partially hepatectomized and fed 0.2% Oil Orange SS in the diet for 10 days (Gershbein 1982).

7.2.2.2.3 Other health effects

A study abstract reported that giving 0.1% or 0.25% Oil Orange SS to rats in the diet (50 or 125 mg/kg-bw per day) for 2 years produced damages in the liver and heart and bile duct enlargement as well as decreases in growth rate and increases in mortality of the treated rats. This abstract also reported that when 16 dogs were fed 0.01–0.2% Oil Orange SS (3–60 mg/kg-bw per day) for 26 days to 2 years, emaciation with internal organ (liver, spleen, lymph nodes, bone marrow, genital organs and skeletal muscle) atrophy was observed at the higher doses (Fitzhugh et al. 1956). When rats were force-fed 200 or 400 mg/kg-bw per day of Oil Orange SS for 20 weeks, significantly decreased hemoglobin levels and increased relative organ weights, including spleen, liver, heart and testis, were observed (Allmark et al. 1965).

When Wistar rats were fed 4% Oil Orange SS in the diet (2000 mg/kg-bw per day) for 3 weeks or longer, rats died of severe gastroenteritis in a few months. Oil Orange SS stained the small intestine of all treated animals and the forestomach and glandular

stomach of some treated animals. Granular deposits of Oil Orange SS in the stomach, small intestine and colon of the treated animals were observed (Willheim and Ivy 1953). In another short-term study, rats were fed 0.1% Oil Orange SS in the diet (50 mg/kg-bw per day) for 10 days; significantly increased relative liver weights were observed, but no other health effects were examined (Gershbein 1982). A single oral dose of 100 or 200 mg Oil Orange SS caused cathartic effects in the treated dogs (Radomski and Deichmann 1956).

Oil Orange SS tested positive for skin sensitization in some human patients (Baer et al. 1948; Kozuka et al. 1980).

7.2.2.2.4 Summary

Overall, available health effects data for this substance are limited. Oil Orange SS induced chromosomal damages *in vivo* and gene mutations in bacteria with metabolic activation and intestinal tumours in mice via oral administration, indicating carcinogenicity and genotoxicity are the main health concerns for Oil Orange SS.

7.2.2.3 Solvent Red 1 (CAS RN 1229-55-6)

7.2.2.3.1 Carcinogenicity and genotoxicity

Solvent Red 1 has been classified by the European Commission as a Category 1B carcinogen – “May cause cancer” (European Commission 2008). Empirical data related to the carcinogenic potential of Solvent Red 1 have not been identified.

The genotoxic potential of Solvent Red 1 has been investigated in one *in vivo* and some *in vitro* experiments. Solvent Red 1 did not induce micronuclei in Swiss mice following intraperitoneal injection (Manthei et al. 1983). Solvent Red 1 induced gene mutations and micronuclei in mouse lymphoma cells *in vitro* with metabolic activation where cytotoxicity was also observed (Moore et al. 1989; Harrington-Brock et al. 1991). Solvent Red 1 weakly induced sister chromatid exchanges, but not gene mutations or chromosomal aberrations, in CHO cells with metabolic activation, which may be associated with cytotoxicity (Henderson et al. 1986; Brooks et al. 1989). Solvent Red 1 exhibited weak mutagenicity in *S. typhimurium* strain TA100 with metabolic activation in some studies (Henry 1983; Moore et al. 1989), but not in others (Manthei et al. 1983; Henderson et al. 1986; Brooks et al. 1989). Equivocal Ames test results in strains TA102 and TA104 and negative results in strains TA98, TA1535, TA1537 and TA1538, with and without metabolic activation, were reported (Henry 1983; Henderson et al. 1986; Brooks et al. 1989; Moore et al. 1989). Under reductive Ames test conditions, negative results in strains TA98, TA100, TA1535 and TA1538 were reported (Brooks et al. 1989).

In addition, Solvent Red 1 stimulated liver regeneration in rats that were partially hepatectomized and fed 0.2% Solvent Red 1 in the diet for 10 days (Gershbein 1982).

7.2.2.3.2 Other health effects

When male Holtzman rats were fed 0.15% Solvent Red 1 in the diet (75 mg/kg-bw per day) for 10 days, the relative liver weights of the treated rats did not change significantly (no other health effects were examined) (Gershbein 1982). In single-day studies, Solvent Red 1 did not cause animal death at dose levels up to 5 g/kg-bw in rats via oral administration and up to 2 g/kg-bw in rabbits via dermal application (Manthei et al. 1983; Smith et al. 1986).

Solvent Red 1 tested positive in the mouse local lymph node assay and modified mouse ear swelling test, suggesting it may have contact-sensitizing potential (Sailstad et al. 1993, 1994). It caused contact dermatitis in a human patient (Wantke et al. 1992).

7.2.2.3.3 Summary

Overall, the empirical data for Solvent Red 1 are limited, particularly owing to the lack of chronic toxicity data and sufficient *in vivo* genotoxicity data to permit a full evaluation on its health effects potential. Limited data indicate that Solvent Red 1 is a weak mutagen in bacteria and mammalian cells, but it has exhibited potent cytotoxicity *in vitro*. In addition, although the azo bond reductive cleavage potential of Solvent Red 1 remains unknown, given the similarity between Solvent Red 1 and Sudan I, it is reasonable to assume that the azo linkage of Solvent Red 1 can be reduced by gut and/or skin bacteria to a certain extent. If this occurred, 1-amino-2-naphthol and *o*-anisidine could be released. *o*-Anisidine is one of the EU22 aromatic amines that has exhibited carcinogenicity and genotoxicity in experimental animals (Environment Canada and Health Canada 2014b).

7.2.2.4 Sudan IV (CAS RN 85-83-6)

7.2.2.4.1 Absorption, distribution, metabolism and excretion

Following intratracheal administration of ^{14}C-labelled Sudan IV in rats, 60% of the applied dose was absorbed into the body, and 98% of the absorbed dose was excreted within 96 hours, mainly in the feces and to a lesser extent in the urine. Traces of radioactivity were found in the liver and other organs and tissues (Parent and Dressler 1979; EFSA 2005). When Sudan IV was instilled in rat lung, 74 ± 20% and 72 ± 15% of the dosed dyes were recovered after 5 minutes and 24 hours, respectively, from the lungs (Henderson et al. 1988). In another study, slightly increased cytochrome P450 enzyme level and UDPGT activity were detected in rats administered Sudan IV via intraperitoneal injection for 4 days (Fujita et al. 1984). Sudan IV was degraded when incubatng with human intestinal microflora under anaerobic conditions, and *o*-toluidine was detected (Xu et al. 2007, 2010). The azo linkage of Sudan IV was rapidly reduced by the skin bacteria *S. epidermidis* and *M. luteus* under aerobic conditions (BRI 2013b).

7.2.2.4.2 Carcinogenicity and genotoxicity

Sudan IV was classified by the European Commission as a Category 2 carcinogen – "Suspected of causing cancer" (European Commission 2008) and by IARC as a Group 3 substance – "Not classifiable as to its carcinogenicity to humans" (IARC 1987a).

The carcinogenicity of Sudan IV via oral administration has been investigated in rats and mice. When heterogeneous mice were fed 2 mg/day of Sudan IV in the diet (67 mg/kg-bw per day) for 635–653 days, lung tumours and lymphomas were observed (2/81 and 7/25 of the treated males and females, respectively, compared with 7/109 and 11/59 of the male and female controls, respectively). One treated female developed liver adenoma (none in the controls). The authors considered that the presence of a carcinogenic action of Sudan IV in this study was doubtful (Waterman and Lignac 1958). In orally dosed rats, hepatomas were observed in 2 of 20 rats that were fed 1000 mg/kg of Sudan IV in the diet (50 mg/kg-bw per day) for life; however, data for the control group were not provided (Hackmann 1951). In another oral cancer bioassay, hyperplasia of the bile ducts, resembling cholangiomas, was observed in one of four Wistar rats that were fed 4% Sudan IV in the diet (2000 mg/kg-bw per day) for 12–18 months (Willheim and Ivy 1953).

IARC (1975h) reviewed several old studies that were conducted via non-conventional routes of exposure. Following dermal application plus subcutaneous injection of Sudan IV, no tumours developed in the treated mice, while hyperplasia of the epithelial layer was observed. Subcutaneous injection of Sudan IV into the mammary gland area of mice did not induce tumours; atypical epithelial proliferation and cystadenomatous proliferation at the injection area were noted. Following subcutaneous injection of Sudan IV, four of eight treated rats developed sarcomas at the injection sites. Subcutaneous injection of Sudan IV in rabbits caused epithelial proliferation. A single injection of Sudan IV into the wall of the urinary bladder did not induce tumours; when two beads of wax dental cement were deposited into the bladder lumen at the same time, bladder carcinoma (1/6) and bladder papilloma (4/6) were observed in the treated rabbits, whereas 3/15 of the control rabbits developed bladder papillomas.

The genotoxic potential of Sudan IV has been investigated *in vitro*. Sudan IV did not induce gene mutations in Ames tests in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, with and without metabolic activation (Brown et al. 1978; Miyagoshi et al. 1985; Henderson et al. 1986). Under reductive Ames test conditions with metabolic activation, positive results were observed in *S. typhimurium* strain TA98 with FMN (Zhou et al. 1987) and in strains TA98, TA1537 and TA1538, but not in strains TA100 and TA1535, with dithionite (Brown et al. 1978).

In addition, Sudan IV significantly stimulated liver regeneration in rats that were partially hepatectomized and fed 0.1% or 0.2% Sudan IV in the diet (50 or 100 mg/kg-bw per day) for 10 days (Gershbein 1982). Sudan IV enhanced human tumour cell proliferation *in vitro* (Ji et al. 2006a, b) and induced SA7/SHE cell transformation (Heidelberger et al. 1983). Sudan IV suppressed DMBA-induced chromosomal aberrations in rat bone

marrow, an effect that was also observed with Sudan I, suggesting that Sudan IV may have induced metabolic enzymes that are related to DMBA detoxification process (Ito et al. 1982).

7.2.2.4.3 Other health effects

Cirrhotic changes were observed in one of four Wistar rats that were fed 4% Sudan IV in the diet (2000 mg/kg-bw per day) for 18 months; Sudan IV-stained forestomach and granular deposits of Sudan IV were also observed (Willheim and Ivy 1953). Significantly increased relative liver weights were observed in rats fed 0.1% or 0.2% Sudan IV in the diet (50 or 100 mg/kg-bw per day) for 5–10 days; no other health effects were examined (Gershbein 1982).

Sudan IV was classified by the European Commission as a Category 1 skin sensitizer – "May cause an allergic skin reaction" (European Commission 2008). Epidermal hyperplasia was observed in guinea pigs following the intradermal injection of Sudan IV (Stenn 1979).

7.2.2.4.4 Summary

Overall, the empirical health effects data for Sudan IV are limited, particularly owing to the lack of adequate chronic toxicity studies and *in vivo* genotoxicity data to permit a full evaluation of its health effects potential. Sudan IV induced gene mutations in bacteria under reductive conditions with metabolic activation. Sudan IV also induced mammalian cell transformation. However, unambiguous evidence of carcinogenic potential of Sudan IV has not been observed.

7.2.3 Miscellaneous Substances

Among the remaining 10 Azo Solvent Dyes (Table 7-4) that were not included in the previous two health subsets, limited empirical data on health effects have been identified for three substances: Solvent Red 3, Solvent Yellow 18 and Solvent Red 19. No empirical data were identified for the remaining seven substances.

Table 7-4. Miscellaneous Substances

Parent substance name (CAS RN)	Poatulated azo bond reductive cleavage products (CAS RN)
4-Anilinoazobenzene (101-75-7)	Aniline (62-53-3) *p*-Aminodiphenylamine (101-54-2)
Solvent Red 4 (2653-64-7)	1-Naphthylamine (134-32-7) 1-Amino-2-naphthol (2834-92-6)
Magneson II (5290-62-0)	4-Nitroaniline (100-01-6) 4-Amino-1-naphthol (2834-90-4)
Solvent Red 19 (6368-72-5)	Aniline (62-53-3) *p*-Phenylenediamine (106-50-3) *p*-Aminoazobenzene (60-09-3) [EU22 aromatic amine] Others, no CAS RN
Solvent Yellow 18 (6407-78-9)	2, 4-Xylidine (95-68-1) Others, no CAS RN

Parent substance name (CAS RN)	Poatulated azo bond reductive cleavage products (CAS RN)
Solvent Red 3 (6535-42-8)	*p*-phenetidine (156-43-4) 1-amino-2-naphthol (2834-92-6)
NA (21519-06-2)	Aniline (62-53-3) *p*-Phenylenediamine (106-50-3) *p*-Aminoazobenzene (60-09-3) [EU22 aromatic amine] Other, no CAS RN
NA (73507-36-5)	*p*-Phenylenediamine (106-50-3) Sulfanilic acid (121-57-3) Others, no CAS RN
NA (73528-78-6)	Dicloran (99-30-9) 4-Amino-2,5-dimethyoxyaniline (17626-02-7) Other, no CAS RN
NA (85392-21-8)	Aniline (62-53-3) 2-Chloro-*p*-phenylenediamine (615-66-7) Other, no CAS RN

7.2.3.1 Solvent Red 3 (CAS RN 6535-42-8)

The carcinogenic potential of Solvent Red 3 was investigated in rats that were fed 1000 mg/kg of Solvent Red 3 in the diet (50 mg/kg-bw per day) for 1 year (19/20 of the treated rats survived) or longer (3/20 survived for 2 years). Most of the animals died from pneumonia or lung abscesses. The pathological changes in the liver were primarily related to necrosis, congestion and fatty degeneration. The liver and stomach/intestinal tract of the treated animals showed no alterations to indicate that this substance was carcinogenic. One rat that survived 764 days (total dose of 10.2 g) developed a fibroadenoma in the breast. No data for the control animals were provided (Hackmann 1951). No empirical data regarding other health effects of Solvent Red 3 or its absorption, distribution, metabolism and/or excretion have been identified.

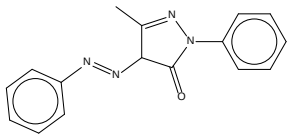
Structurally, Solvent Red 3 is similar to Sudan I, except that it does not have the *ortho*-hydroxyl- group to form a coplanar structure, which is a characteristic of Sudan Dyes. This leads to uncertainty as to whether it can undergo metabolic activation pathways similar to that of Sudan I. It is also uncertain as to what extent its azo linkage undergoes cleavage under *in vivo* conditions and how its azo reductive cleavage products influence its health effects. If the azo linkage of Solvent Red 3 were cleaved, *p*-phenetidine and 4-amino-1-naphthol could be released. The health effects of *p*-phenetidine have been evaluated in the Screening Assessment on Certain Aromatic Amines (Environment Canada and Health Canada 2014b). There are some evidence indicating that *p*-phenetidine induced gene mutations in bacteria and mammalian cells, chromosomal damages *in vivo* and *in vitro* and DNA damages *in vitro*. While *in vivo* cancer bioassays for *p*-phenetidine have not been identified, it tested negative in mouse embryo cell transformation *in vitro*. For 4-amino-1-naphthol, minimal health effects information is available. One study reported positive Ames test results with metabolic activation (Freeman et al. 1987). Another study reported that 4-amino-1-naphthol generated reactive oxygen species *in vitro* (Nakayama et al. 1983), suggesting that it has cytotoxicity potential.

Overall, the limited health effects information provided by the available chronic toxicity study does not indicate a carcinogenic potential of Solvent Red 3.

7.2.3.2 Solvent Yellow 18 (CAS RN 6407-78-9)

The mutagenicity of Solvent Yellow 18 has been tested in one Ames test in *S. typhimurium* strains TA98 and TA100 under standard and reductive (with FMN) conditions and with metabolic activation. Positive results were observed in strain TA98 with and without FMN, and the results were negative in strain TA100 (ILS 2011). When incubating with skin bacteria *S. epidermidis* and *M. luteus* under aerobic conditions, the azo linkage of Solvent Yellow 18 was reduced within 6 hours, and only an unknown metabolite was detected (BRI 2013b); another postulated azo bond cleavage product of Solvent Yellow 18, 2, 4-xylidine, for which there are concerns about potential cancer and hematological effects (OECD 2012), was not detected. Under anaerobic conditions, only minimal azo bond cleavage of Solvent Yellow 18 was observed over 24-hour incubation with human fecal microflora (BRI 2013a).

Appropriate analogue for Solvent Yellow 18 has not been identified. There are limited health effects data available for Sudan Yellow 3G (also known as Solvent Yellow 16, CAS RN 4314-14-1), which is structurally similar to Solvent Yellow 18, except that Sudan Yellow 3G has an aniline moiety in the place of 2,4-xylidine moiety in Solvent Yellow 18.



Sudan Yellow 3G
CAS RN 4314-14-1

One study reported that “feeding Sudan Yellow 3G for 61 weeks to mice (0.1% and 1% in diet, 13–130 mg/kg-bw per day) did not cause significant changes in the morphology, composition of blood, haemoglobin value, erythrocytes or organs” (Majlathova and Rippel 1970). The Scientific Committee on Cosmetology (SCC 1988) reported two oral repeated-dose studies. A 6-week oral study in rats (100 or 500 mg/kg-bw per day via gavage) did not reveal growth depression or gross or microscopic changes of the heart, lungs, liver, kidneys, adrenals or spleen. In a long-term study, tumour development was not observed in rats fed the colourant mixed in the diet at 60 mg/kg (3 mg/kg-bw per day); 19 of 20 treated rats survived for a year, and 2 of 20 survived for 2 years. The quality of these studies cannot be evaluated as only limited information is available. Hence, the critical health effect levels could not be defined for these studies for data read-across purposes for Solvent Yellow 18.

Overall, available data on Sudan Yellow 3G have not provided evidence to indicate a high hazard potential of Solvent Yellow 18.

7.2.3.3 Solvent Red 19 (CAS RN 6368-72-5)

Solvent Red 19 (also known as Sudan Red 7B) is classified by IARC as a Group 3 substance– “Not classifiable as to its carcinogenicity to humans” (IARC 1987a). Only two dated cancer assays were identified for this substance (IARC 1975j). A group of 75 rats was fed 1000 mg/kg of Solvent Red 19 in the diet (28.6–42.9 mg/kg-bw per day) for up to 350 days. One hepatoma and one cecal tumour were found; however, the survival rates of the treated animals and the data on the controls were not reported (IARC 1975j). In another study, rats were fed 1000 mg/kg of Solvent Red 19 in the diet (50 mg/kg-bw per day) for life. Nineteen rats survived 1 year, and five rats survived more than 2 years. No tumours or pathological changes in the liver, stomach or intestine of the treated rats were observed (Hackmann 1951).

Several studies conducted after the publication of the IARC (1975j) assessment have investigated the genotoxic potential of Solvent Red 19. Solvent Red 19 did not cause DNA damages in the stomach, colon, liver, kidney, bladder, lung, brain or bone marrow tissues, as measured by the comet assay, in orally dosed ddY mice (Tsuda et al. 2000). Solvent Red 19 induced gene mutations in both bacteria and mammalian cells. Positive results were observed in Ames tests in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 and in the mouse lymphoma assay with metabolic activation (Litton Bionetics Inc. 1982).

Solvent Red 19 did not induce BALB/3T3 cell transformation in the absence of metabolic activation (Litton Bionetics Inc. 1982).

A recent epidemiological investigation studied nine bladder cancer patients who had worked with dye penetrants (crack testers) at their previous workplace. The crack testers contain Sudan Red (the authors identified it as Sudan Red 7B) and *p*-phenylazo-anilin-*N*-ethyl-2-naphthylamine. A causal relationship between Solvent Red 19 and bladder cancer risk in the workers was not established (Golka et al. 2012). Another epidemiological investigation studied bladder cancer patients who were working with dye penetrants to detect metal cracks. Solvent Red 164 was reported to be in the dye penetrants (Noon et al. 2012). The chemical composition of this dye contains 2-naphthenol [(phenylazo)phenyl] azo alkyl derivatives (Apex Oil Company, Inc. 2007), which may include Solvent Red 19.

Solvent Red 19 did not effectively induce cytochrome P450 or UDPGT activity in rats following four intraperitoneal injections of 40 mg/kg-bw per day of Solvent Red 19 in corn oil (Fujita et al. 1984).

Overall, Solvent Red 19 is considered to have mutagenic potential. Although available data have not provided carcinogenic evidence of this substance, *p*-aminoazobenzene, an EU aromatic amine that has produced carcinogenic effects in experimental animals, could potentially be released from this substance through azo bond reductive cleavage.

7.2.3.4 Substances with no empirical health effects data

No empirical health effects data have been identified for the remaining seven Azo Solvent Dyes in this subset. Magneson II and CAS RN 73528-78-6 may have genotoxic potential due to the presence of aromatic nitro group in their structures. In general, all substances have the potential to undergo reductive cleavage of the azo bond, which may lead to the release of carcinogenic metabolites. However, there is uncertainty with respect to the extent of azo bond reduction and the actual metabolites released *in vivo*.

7.2.4 Uncertainty

The confidence in the health effects assessment for Azobenzene and Its Derivatives is relatively high, as these substances, except for Solvent Orange 3, have been extensively studied. The confidence for Sudan Dyes assessment is moderate, as Sudan I is the only substance that has been extensively investigated. Although the metabolic activation pathways of Sudan I could also be potentially applied to its analogues, the relative toxicity potency among these analogues remains unclear. The confidence for the Miscellaneous Substances assessment is low, particularly for those without empirical data. Although some potential metabolic activation pathways leading to the generation of active metabolites have been indicated, the significance of these potential metabolites to the final health effects outcome of the parent substances remains uncertain.

There is uncertainty associated with the test substance identity, particularly for Solvent Orange 3, as it is often unclear in the study reports whether Solvent Orange 3 or Basic Orange 2 was tested, resulting in uncertainty in the corresponding dose-response. Some toxicological tests used commercial dyes that may contain impurities. These impurities may have contributed to some of the health effects observed. It is also recognized that commercial dyes may contain different impurities, resulting in additional health concerns.

There is also uncertainty in the activation pathways for azo solvent dyes as these substances can be absorbed directly or be biodegraded to aromatic amines first and subsequently absorbed. These pathways lead to various active metabolites. As such, there is uncertainty regarding how the postulated azo bond reductive cleavage products contribute to the health effects outcome of the parent substances as the rate and extent of azo bond reduction remain unknown.

In addition, many toxicological studies focused only on carcinogenic effects of the Azo Solvent Dyes; in general, other health effects, such as reproductive and developmental effects, have not beenwell studied.

7.3 Characterization of Risk to Human Health

The focus of the human health risk characterization was on those Azo Solvent Dyes to which exposure of the general population of Canada was indicated (refer to Exposure

Assessment section). Exposure to the 20 Azo Solvent Dyes through environmental media (i.e., air, water, soil, sediment) and food is not expected; therefore risk to human health from these exposure sources is not expected.

For some Azo Solvent Dyes, their use in products available to consumers in the Canadian marketplace has not been reported or indicated. Exposure to these substances (*p*-aminoazobenzene, Solvent Yellow 2, Solvent Yellow 3, 4-anilinoazobenzene, azobenzene, Oil Orange SS, Solvent Red 4, Magneson II, Solvent Red 19, CAS RN 21519-06-2, CAS RN 73507-36-5, CAS RN 73528-78-6 and CAS RN 85392-21-8) is not expected. For other substances (Solvent Orange 3, Solvent Yellow 77, Sudan I, Solvent Red 1, Sudan IV, Solvent Red 3 and Solvent Yellow 18), where their use in certain products available to consumers has been identified, exposure to these substances has been estimated or considered for the general population of Canada for relevant exposure routes and durations.

7.3.1 Azobenzene and Its Derivatives

Based on the empirical data identified, the critical health effects associated with exposure to Azobenzene and Its Derivatives (i.e., azobenzene, *p*-aminoazobenzene, Solvent Yellow 2, Solvent Orange 3, Solvent Yellow 3 and Solvent Yellow 77) are considered to be carcinogenicity and genotoxicity. In addition, *p*-aminoazobenzene, Solvent Yellow 2, azobenzene and Solvent Yellow 77 also caused hematological effects in experimental animals.

7.3.1.1 Solvent Orange 3

Exposure to Solvent Orange 3 from use of shoe polish products was estimated to be 0.021 mg/kg-bw per event for the dermal route. Available empirical data on Solvent Orange 3 or Basic Orange 2-induced health effects during shorter durations of exposure are limited (Environment Canada and Health Canada 2014b). However, hematological effects were observed in rats that received chrysoidine in drinking water for 21 days at a dose level (total 670 mg/rat) that is three orders of magnitude greater than the estimated exposure level for use of shoe polish products. Therefore, risk to human health through skin contact with shoe polish products containing Solvent Orange 3 is considered to be low.

7.3.1.2 Solvent Yellow 77

Exposures to Solvent Yellow 77 for the dermal route were estimated to range from 5.5×10^{-4} to 8.7×10^{-4} mg/kg-bw per day for daily use of textile clothing and 4.4×10^{-4} to 1.7×10^{-2} mg/kg-bw for use of leather products. Exposure to Solvent Yellow 77 due to mouthing of textiles by infants was estimated to be 2.7×10^{-5} mg/kg-bw per day.

In the absence of dermal health effects data for Solvent Yellow 77, the following oral effect levels were used to derive the MOEs for both oral and dermal routes: a lowest $BMDL_{10}$ of 51.29 mg/kg-bw per day, based on Solvent Yellow 77–induced liver tumours

in rats; a chronic LOEL of 250 mg/kg-bw per day, based on Solvent Yellow 77–induced pigmentation in the kidneys, body weight gain reduction and focal hepatic cellular changes in rats; and a range of oral short-term NOAELs of 1250–1625 mg/kg-bw per day, based on Solvent Yellow 77–induced body weight gain depression in rodents (NTP 1982a). In the NTP study (1982a), the test material contains 87.6 % Solvent Yellow 77. Considering the dyes used in textiles and leather products are also commercially available dyes that may not contain 100% Solvent Yellow 77, further dosimetric adjustment was not conducted for this study as well as for other toxiclogical studies that used commercial dyes.

Comparison of the exposure estimates for mouthing of textile clothing with the oral $BMDL_{10}$ and the chronic LOEL results in MOEs of 190 000 and 930 000 for potential cancer and non-cancer effects, respectively. These MOEs are considered adequate to address uncertainties in the health effects and exposure databases (Table 7-5).

Comparison of the exposure estimates for skin contact with textile clothing with the oral $BMDL_{10}$ and chronic LOEL results in MOEs ranging from 60 000 to 90 000 for potential cancer effects and from 280 000 to 450 000 for potential non-cancer effects (Table 7-5). These MOEs are considered adequate to address uncertainties in the health effects and exposure databases for both cancer and critical non-cancer effects. Comparison of the exposure estimates for skin contact with leather products with the oral short-term NOAEL results in MOEs ranging from 74 000 to 3 693 000. These MOEs are considered adequate to address uncertainties in the health effects and exposure databases (Table 7-5).

It is also recognised that young children (predominately 0.5 – 4 years of age) may incidentally ingest paper products that contain Solvent Yellow 77. However, empirical data do not indicate that acute toxicity is a health concern for Solvent Yellow 77. Therefore, risk to young children from exposure to paper products containing Solvent Yellow 77 is expected to be low.

Table 7-5. Summary of MOEs derived for use of consumer products containing Solvent Yellow 77

Exposure scenario[a]	Exposure estimates	Critical health effect level (mg/kg-bw per day)	MOE
Mouthing of textile objects by infants (oral)	2.7×10^{-5} Daily (mg/kg-bw per day)	Oral $BMDL_{10}$ = 51.29 (rat)	190 000
Mouthing of textile objects by infants (oral)	2.7×10^{-5} Daily (mg/kg-bw per day)	Oral chronic LOAEL = 250 (rat)	930 000
Textiles: Personal apparel worn by adults, Baby sleeper (dermal)	5.5×10^{-4} to 8.7×10^{-4} Daily (mg/kg-bw per day)	Oral $BMDL_{10}$ = 51.29 (rat)	60 000 – 90 000
Textiles: Personal apparel worn by adults, Baby sleeper (dermal)	5.5×10^{-4} to 8.7×10^{-4} Daily (mg/kg-bw per day)	Oral chronic LOAEL = 250 (rat)	280 000 – 450 000

Leather products (dermal)	4.4×10^{-4} to 1.7×10^{-2} Per event (mg/kg-bw)	Oral short-term NOAELs = 1250–1625 (mouse, rat)	74 000 – 3 693 000

Abbreviations: $BMDL_{10}$, lower confidence limit on the benchmark dose for a 10% response; LOAEL, lowest-observed-adverse-effect level; MOE, margin of exposure; N/A, not applicable; NOAEL, no-observed-adverse-effect level
[a] Exposure scenarios consider adults 20–59 years of age unless otherwise specified.

7.3.2 Sudan Dyes

Based on the empirical data identified for the Sudan Dyes and data from read-across, potential for carcinogenicity and genotoxicity as well as potential for causing hematological effects are recognized for the Sudan Dyes (i.e., Sudan I, Oil Orange SS, Solvent Red 1 and Sudan IV), although the toxic potency of individual substances varies.

7.3.2.1 Sudan I

Per event exposures to Sudan I from use of writing ink due to age-related hand-to-mouth behaviours in children were estimated for both oral and dermal routes. The resulting exposure estimates range from 1.6×10^{-3} to 8.1×10^{-3} mg/kg-bw for the oral route and from 4.2×10^{-4} to 2.1×10^{-3} mg/kg-bw for skin contact.

Sudan I- induced health effects during short-term exposure were observed in oral studies. Severe health effects including liver congestion and intestine discolouration were observed in rats and mice at dose level of 300 mg/kg-bw per day (the lowest dose tested; the test material purity was 94.1%; further dosimetric adjustment was not conducted on the aforementioned ground) and above (NTP 1982b). At a lower dose level (25 mg/kg-bw per day), significantly increased relative liver weights were observed in treated rats (Gershbein 1982), although the toxicological significance of this finding is limited owing to the lack of dose-response data (only one dose level was used in the study) and no other potential health effects were examined. Therefore, the point of departure (POD) for risk characterization for the oral exposure from use of writing ink is considered to be in the range of 25 to 300 mg/kg-bw per day. In the absence of dermal health effects data for Sudan I, the oral POD was used to derive MOEs for both oral and dermal routes. Comparison of the exposure estimates from use of writing ink with the oral short-term POD results in MOEs ranging from 3 000 to 188 000 for the oral route and from 12 000 to 714 000 for the dermal route. These MOEs are considered adequate to address uncertainties in the health effects and exposure databases (Table 7-6).

7.3.2.2 Solvent Red 1

Exposure to Solvent Red 1 from use of certain cosmetic products was estimated for the dermal route. The exposure estimates for skin contact with various cosmetic products range from 3.7×10^{-5} mg/kg-bw per day to 5.3×10^{-4} mg/kg-bw per day.

Health effects information for Solvent Red 1 is limited. Chronic toxicity data for Solvent Red 1 have not been identified. In the absence of appropriate data and as a conservative approach, data read-across from Sudan I to Solvent Red 1 was applied. As health effects data via the dermal route have not been identified for Sudan I, a lowest oral $BMDL_{10}$ of 5.54 mg/kg-bw per day, based on Sudan I–induced neoplastic nodules in the liver of the treated rats, and an oral chronic LOAEL of 12.5 mg/kg-bw per day, based on Sudan I–induced systemic effects (cardiac valve multifocal fibrosis, lung lymphoid hyperplasia, bile duct focal hyperplasia, pancreatic acinus atrophy and nephropathy) (NTP 1982b), were used to derive MOEs for Solvent Red 1 for the dermal route.

Comparison of the exposure estimates for the dermal route for the use of hair conditioner, hair straightener, shower soap or manicure preparation gel with the lowest oral $BMDL_{10}$ and the chronic LOAEL results in MOEs ranging from 10 000 to 150 000 for potential cancer effects and from 24 000 to 338 000 for non-cancer effects (Table 7-6). These MOEs are considered adequate to address uncertainties in the health effects and exposure databases.

Table 7-6. Summary of MOEs derived for use of consumer products and cosmetics containing Sudan I or Solvent Red 1

Substance	Exposure scenario[a]	Exposure estimates	Critical health effect level (mg/kg-bw per day)	MOE
Sudan I	Incidental ingestion of writing ink[b] (toddler; oral)	1.6×10^{-3} to 8.1×10^{-3} Per event (mg/kg-bw)	Oral short-term effect levels = 25 - 300 (rat)	3 000 – 188 000
Sudan I	Writing ink (toddler; dermal)	4.2×10^{-4} to 2.1×10^{-3} Per event (mg/kg-bw)	Oral short-term effect levels = 25 - 300 (rat)	12 000 – 714 000
Solvent Red 1	Hair conditioner, hair straightener, shower soap, manicure preparation gel (dermal)	3.7×10^{-5} to 5.3×10^{-4} Daily (mg/kg-bw per day)	Oral $BMDL_{10}$ = 5.54 (rat)	10 000 – 150 000
Solvent Red 1	Hair conditioner, hair straightener, shower soap, manicure preparation gel (dermal)	3.7×10^{-5} to 5.3×10^{-4} Daily (mg/kg-bw per day)	Oral chronic LOAEL = 12.5 (rat)	24 000 – 338 000

Abbreviations: $BMDL_{10}$, lower confidence limit on the benchmark dose for a 10% response; LOAEL, lowest-observed-adverse-effect level; MOE, margin of exposure; N/A, not applicable

[a] Exposure scenarios consider adults 20–59 years of age unless otherwise specified.

[b] Via hand-to-mouth activity.

7.3.2.3 Sudan IV

Sudan IV was identified to be used in food packaging materials; however, minimal potential for direct food contact is expected in this application. Therefore, risk to human health from exposure to Sudan IV is considered to be low.

7.3.3 Miscellaneous Substances

Owing to the paucity of empirical data, the critical health effects for the 10 solvent dyes in the Miscellaneous Substances subset (i.e., 4-anilinoazobenzene, Solvent Red 4, Magneson II, Solvent Red 19, Solvent Yellow 18, Solvent Red 3, CAS RN 21519-06-2, CAS RN 73507-36-5, CAS RN 73528-78-6 and CAS RN 85392-21-8) were not conclusively determined. Although *in vitro* data showed that Solvent Red 19 and Solvent Yellow 18 caused gene mutations, the *in vivo* genotoxic potential of Solvent Yellow 18 has not been investigated, and the results of the only available *in vivo* genotoxicity testing for Solvent Yellow 19 were negative. In addition, Magneson II and CAS RN 73528-78-6 may have genotoxic potential due to the aromatic nitro group present in their structures.

7.3.3.1 Solvent Red 3

Exposure to Solvent Red 3 from the use of certain cosmetic products was characterized for both dermal and inhalation routes. The exposure estimates for skin contact with various cosmetic products range from 9.6×10^{-5} to 2.1×10^{-3} mg/kg-bw per day and the inhalation intake from spray perfume was 1.2×10^{-6} mg/kg-bw per day.

Based on limited health effects data for Solvent Red 3 and its postulated azo bond cleavage products, there is no evidence to indicate that Solvent Red 3 might be carcinogenic. Solvent Red 3 is structurally similar to Sudan I, although it was not considered to be a member of the Sudan Dyes (refer to the Human Health Effects Assessment). In the absence of appropriate data for Solvent Red 3 and as a conservative approach, an oral chronic LOAEL of 12.5 mg/kg-bw per day based on health effects data for Sudan I was used to derive the MOEs for Solvent Red 3 for both dermal and inhalation routes.

Comparison of the exposure estimates for skin contact from spray perfume, essential oil depilatory cream or shower soap with the oral chronic LOAEL results in MOEs ranging from 6 000 to 6 500 for potential chronic effects. Comparison of the exposure estimate for inhalation from spray perfume with the oral chronic LOAEL results in MOE of 1×10^{7} for potential chronic effects. These MOEs are considered adequate to address uncertainties in the health effects and exposure databases (Table 7-7).

Table 7-7. Summary of MOEs derived for use of cosmetics containing Solvent Red 3

Exposure scenario[a]	Exposure estimates	Critical health effect level (mg/kg-bw per day)	MOE

Exposure scenario[a]	Exposure estimates	Critical health effect level (mg/kg-bw per day)	MOE
Spray perfume, essential oil, depilatory cream, shower soap (dermal)	2.1×10^{-3} to 1.9×10^{-3} Daily (mg/kg-bw per day)	Oral chronic LOAEL= 12.5 (rat)	6 000 – 6 500
Spray perfume (inhalation)[a]	1.2×10^{-6} Daily (mg/kg-bw per day)	Oral chronic LOAEL= 12.5 (rat)	1×10^{7}

Abbreviations: LOAEL, lowest-observed-adverse-effect level; MOE, margin of exposure; N/A, not applicable
[a] Exposure scenarios consider adults 20–59 years of age unless otherwise specified.

7.3.3.2 Solvent Yellow 18

Exposure to Solvent Yellow 18 from the use of certain cosmetic products was characterised for the dermal route. The exposure estimates for skin contact with leave-in hair conditioner, hair conditioner and shower soap containing Solvent Yellow 18 range from 7.7×10^{-5} to 5.3×10^{-3} mg/kg-bw per day.

Based on the limited health effects information for Solvent Yellow 18, MOEs for the use of this substance in cosmetic products were not derived. However, read-across to health effects for a structurally similar substance Sudan Yellow 3G (see Section 7.2.3.2), does not indicate a high concern for potential toxicity of Solvent Yellow 18. Therefore, risk to human health from use of products containing Solvent Yellow 18 is considered to be low.

7.3.4 Uncertainties in Evaluation of Risk to Human Health

Appropriate epidemiological studies in relation to human health effects and exposure to Azo Solvent Dyes have not been identified. Some epidemiological investigations suggested an association between human bladder cancer risk and the use of certain products containing Azo Solvent Dyes; however, the causality between azo solvent dye exposure and bladder cancer risk cannot be established from these investigations. This leads to uncertainty with respect to the human relevance of the health effects observed in experimental animals, which served as the basis for the risk characterization for these dyes. However, some Azo Solvent Dyes have exhibited clear evidence of genotoxicity *in vivo* and/or *in vitro*. They may also induce gene mutation and/or cancer in humans as the metabolic enzymes for the activation of these dyes as well as bacterial-mediated azo bond reduction are commonly present in both humans and experimental animals. However, there may be toxicodynamic and toxicokinetic differences across species.

In the absence of health effects data via the dermal and inhalation routes of exposure, a route-to-route extrapolation approach was used throughout the quantitative risk characterization, which may result in some uncertainties, as there may be differences in the absorption rate and ratio as well as metabolism by different routes. For substances with limited data, where a toxicological data read-across approach was applied, there is

relatively high uncertainty in the risk characterization, as the health effects potency may vary among different Azo Solvent Dyes.

Uncertainty is recognized with respect to postulated azo bond cleavage products that may be generated during the use of products containing these Azo Solvent Dyes, which may cause additional health concerns.

Solvent Yellow 77 is also recognized for inducing skin allergic reaction, which depends largely on individual susceptibility. For this substance, lifetime-adjusted doses were not specifically calculated for dermal exposure via textile clothing for infants. Estimates of child and adult exposures were less than 2-fold higher than the infant exposure estimate; therefore, a time-weighted exposure estimate would be expected to be within the range provided in this assessment report. Additionally, no adjustments were made to account for the increased susceptibility of infants and children based on quantitative differences in toxicodynamic processes relative to adults.

7.3.5 Azo Solvent 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 in previous sections, some of the Azo Solvent Dyes in this assessment have effects of concern based on potential carcinogenicity. A list of these substances is shown in Appendix I.

8. Conclusion

Considering all available lines of evidence presented in this Screening Assessment, there is low risk of harm to organisms and the broader integrity of the environment from the 21 Azo Solvent Dyes evaluated in the ecological assessment. It is concluded that these Azo Solvent Dyes do not meet the criteria under paragraphs 64*(a)* or 64(*b*) of CEPA 1999, as they are not entering the environment in a quantity or concentration or under conditions that have or may have an immediate or long-term harmful effect on the environment or its biological diversity or that constitute or may constitute a danger to the environment on which life depends.

Based on the information presented in this Screening Assessment, it is concluded that the 19 Azo Solvent Dyes evaluated in the human health assessment do not meet the criteria under paragraph 64(*c*) of CEPA 1999 as they are not entering the environment in a quantity or concentration or under conditions that constitute or may constitute a danger in Canada to human life or health. Accordingly, there are no changes to the conclusions previously made on Solvent Red 1, Solvent Red 3 and Solvent Yellow 18 with respect to the criteria under paragraph 64(*c*) of CEPA 1999.

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

While it was determined that CAS RN 2832-40-8 (Solvent Yellow 77, Disperse Yellow 3) did not pose a risk to human health, the section 64 conclusions of CEPA 1999 for this substance are included in the Azo Disperse Dyes assessment.

The conclusion previously made under the Challenge Initiative that Solvent Red 23 meets criteria set out in paragraph 64*(c)* of CEPA 1999 remains unchanged.

References

Aalto-Korte K, Alanko K, Kuuliala O, Jolanki R. 2007. Late reactions in patch tests: a 4-year review from a clinic of occupational dermatology. Contact Dermatitis 56:81–86.

Abe S. 1984a. [Metabolic activation of chemicals and their inducibility of SCEs]. Tokishikoroji Foramu 7:371–383. [in Japanese].

Abe S. 1984b. SCE induction by indirect mutagens/carcinogens in metabolically active cultured mammalian cell lines. Basic Life Sci 29B:535–545.

Abe S, Sasaki M. 1977. Chromosome aberrations and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals. J Natl Cancer Inst 58:1635–1641.

Abe S, Sasaki M. 1982. Induction of sister-chromatid exchanges by indirect mutagens/carcinogens in cultured rat hepatoma and esophageal tumor cells and in Chinese hamster DON cells co-cultivated with rat cells. Mutat Res 93:409–418.

AccuStandard, Inc. 2008. Material Safety Data Sheet: DYE-044N (Solvent Red 19, CAS RN 6368-72-5) [Internet]. New Haven (CT): MSDSonline. [cited April 2011]. [restricted access]

ACD/Percepta [Prediction Module]. ©1997–2012. Toronto (ON): Advanced Chemistry Development. [cited March 2013]. Available from: www.acdlabs.com/products/percepta/

[ACGIH] American Conference of Governmental Industrial Hygienists, Inc. 1991Documentation of the Threshold Limit Values and Biological Exposure Indices. 6th ed. Volumes I, II, III. Cincinnati, OH: p. 1743

Acros Organics N.V. 2008a. Material Safety Data Sheet: Fast garnet gbc base, 97% (Solvent Yellow 3, CAS RN 97-56-3) [Internet]. Fair Lawn (NJ): MSDSonline. [cited April 2011]. [restricted access]

Acros Organics N.V. 2008b. Material Safety Data Sheet: 4-(4-Nitrophenylazo)-1-naphthol, 99% (Magneson II, CAS RN 5290-62-0) [Internet]. Fair Lawn (NJ): MSDSonline. [cited April 2011]. [restricted access]

Akamatsu Y, Ikegami R. 1968. Induction of hepatoma and systemic amyloidosis in mice by 4-(dimethylamino)azobenzene feeding. Gann 59:201–206.

Akamatsu Y, Takemura T, Ikegami R, Takahashi A, Miyajima H. 1967. Growth behavior of hepatomas in *o*-aminoazotoluene-treated mice in comparison with spontaneous hepatomas. Gann 58:323–330.

Alberta Environment. 2009. Guidelines for the application of municipal wastewater sludges to agricultural lands. March 2001 (original date 2001; updated 2009). Edmonton (AB): Alberta Environment. Available from: http://environment.gov.ab.ca/info/library/6378.pdf

Aldon Corporation. 2010. Material Safety Data Sheet: Solvent Red 23 (85-86-9) [Internet]. Avon (NY): MSDSonline. [cited April 2011]. [restricted access]

[Alfa Aesar] Interactive Alfa Aesar Database [database on the Internet]. ©2011. Ward Hill (MA): Alfa Aesar. [cited April 2011]. Available from: http://www.alfa.com/en/GP100W.pgm?DSSTK=A11277&rnd=058757108

Allmark MG, Grice HC, Mannell WA. 1956. Chronic toxicity studies on food colours. II. Observations on the toxicity of FD&C Green No. 2 (light green SF yellowish), FD&C Orange No. 2 (orange SS) and FD&C Red No. 32 (oil red XO) in rats. J Pharm Pharmacol 8:417–424.

Amacher DE, Turner GN. 1982. Mutagenic evaluation of carcinogens and noncarcinogens in the L5178Y/TK assay utilizing post-mitochondrial fractions (S9) from normal rat liver. Mutat Res 97:49–65.

Ames BN, Durston WE, Yamasaki E, Lee FD. 1973. Carcinogens are mutagens: a simple test system combining liver homogenates for activation and bacteria for detection. Proc Natl Acad Sci USA 70:2281–2285.

An Y, Jiang L, Cao J, Geng C, Zhong L. 2007. Sudan I induces genotoxic effects and oxidative DNA damage in HepG2 cells. Mutat Res 627:164–170.

Anderson D, Styles JA. 1978. An evaluation of 6 short-term tests for detecting organic chemical carcinogens. Appendix II: The bacterial mutation test. Br J Cancer 37:924–930.

Anderson FA. 1996. Final report on the safety assessment of Disperse Yellow 3. J Am Coll Toxicol 15(4):311–319.

Andervont HB. 1942. Induction of hepatic lesions, hepatomas, pulmonary tumors, and hemangio-endotheliomas in mice with *o*-amino-azotoluene. J Natl Cancer Inst 3:131–153.

Andervont HB. 1944. Effect of two azo compounds when added to the diet of mice. J Natl Cancer Inst 4:583–586.

Andervont HB. 1950. Induction of hemangio-endotheliomas and sarcomas in mice with *o*-aminoazotoluene. J Natl Cancer Inst 10:927–941.

Andervont HB. 1958. Induction of hepatomas in strain C3H mice with 4-*o*-tolylazo-*o*-toluidine and carbon tetrachloride. J Natl Cancer Inst 20:431–438.

Andervont HB, Edwards JE. 1943a. Carcinogenic action of two azo compounds in mice. J Natl Cancer Inst 3:349–354.

Andervont HB, Edwards JE. 1943b. Response of strain A female mice to small amounts of *o*-aminoazotoluene. J Natl Cancer Inst 3:355–358.

Anliker R, Moser P, Poppinger D. 1988. Bioaccumulation of dyestuffs and organic pigments in fish: relationships to hydrophobicity and steric factors. Chemosphere 17(8):1631–1644.

[AOPWIN] Atmospheric Oxidation Program for Windows [Estimation Model]. 2010. Version 1.92a. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

Apex Oil Company, Inc. 2007. Material Safety Data Sheet: Dye Solvent Red 164 [Internet]. St. Louis (MO): Apex Oil Company, Inc. Available from: www.apexoil.com/msdsdsr.pdf

Arni P, Mueller D. 1981. Studies on the effectiveness of various metabolizing systems in mutagenicity tests with microorganisms. Mutat Res 85:257–258.

Arnot JA, Arnot M, Mackay D, Couillard Y, MacDonald D, Bonnell M, Doyle P. 2010. Molecular size cut-off criteria for screening bioaccumulation potential: fact or fiction? Integr Environ Assess Manag 6(2):210–224.

Arzamastsev V. 1970. [Change in oxidation–reduction processes in rat tissues during the administration of *o*-aminoazotoluene]. Tr Orengurg Med Inst 20:115–118. [in Russian].

Ashby J, Styles JA, Paton D. 1978. *In vitro* evaluation of some derivatives of the carcinogen Butter Yellow: implications for environmental screening. Br J Cancer 38:34–50.

ASTreat [sewage treatment plant removal model]. 2006. Version 1.0. Cincinnati (OH): Procter & Gamble Company. Available from Procter & Gamble Company, P.O. Box 538707, Cincinnati, OH, 45253-8707, US.

Aterman K. 1987. Localized hepatocarcinogenesis: the response of the liver and kidney to implanted carcinogens. J Cancer Res Clin Oncol 113:507–538.

Baba T, Takayama S. 1961. Influence of *p*-dimethylaminoazobenzene (DAB) pretreatment in neonatal stage on the DAB hepatocarcinogenesis in the adult rats, with special reference to sex difference in the carcinogenesis. Gann 52:73–82.

Baer RL, Leider M, Mayer RL. 1948. Possible eczematous cross-hypersensitivity between paraphenylenediamine and azo-dyes certified for use in foods, drugs and cosmetics. Proc Soc Exp Biol Med 67:489–494.

Bajaj AK, Misra A, Misra K, Rastogi S. 2000. The azo dye Solvent Yellow 3 produces depigmentation. Contact Dermatitis 42:237–238.

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

Barnea ER, Avigdor S. 1990. Coordinated induction of estrogen hydroxylase and catechol-*O*-methyl transferase by xenobiotics in first trimester human placental explants. J Steroid Biochem 35:327–331.

Barnea ER, Avigdor S. 1991. Aryl hydrocarbon hydroxylase activity in the first-trimester human placenta: induction by carcinogens and chemoprotectors. Gynecol Obstet Invest 32:4–9.

Barnea ER, Avigdor S, Boadi WY, Check JH. 1993. Effect of xenobiotics on quinone reductase activity in first trimester explants. Hum Reprod 8:102–106.

Bartek MJ, LaBudde JA, Maibach HI. 1972. Skin permeability *in vivo*: comparison in rat, rabbit, pig and man. J Invest Dermatol 58(3):114–123.

Bartlett A. 2014. Toxicity summary: Effects of Disperse Yellow 7, Sudan Red G, Disperse Orange 13, Acid Red 97, Bismarck Brown Y and Direct Black 38 on the survival and growth of the freshwater amphipod *Hyalella azteca* in chronic, water-only exposures. Internal report (unpublished) prepared by the Aquatic Contaminants Research Division, Water Science and Technology Directorate, Environment Canada. Submitted to the Emerging Priorities Division, Science and Risk Assessment Directorate, Environment Canada. January, 2014. 37p.

Baughman GL, Perenich TA. 1988a. Fate of dyes in aquatic systems: I. Solubility and partitioning of some hydrophobic dyes and related compounds. Environ Toxicol Chem 7:183–199. As cited in EPI Suite 2012.

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

Baughman GL, Weber EJ. 1991. Estimation of water solubility and octanol/water partition coefficient of hydrophobic dyes. Part I: relationship between solubility and partition coefficient. Dyes and Pigments 16:261–271.

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

Bayer AG. 1993. Unpublished results. Report No. 220020, Study No. T2040792. [cited in BUA 2000].

Bedello PG, Goitre M, Cane D. 1982. Contact dermatitis and flare from food flavouring agents. Contact Dermatitis 8:143–144.

[BfR] Bundesinstitut für Risikobewertung. 2007. Introduction to the problems surrounding garment textiles. Berlin (DE): German Federal Institute for Risk Assessment (BfR). Report No.: BfR Information No. 018/2007, 1 June 2007. Available from: www.bfr.bund.de/cm/349/introduction_to_the_problems_surrounding_garment_textiles.pdf

[BIOWIN] Biodegradation Probability Program for Windows [Estimation Model]. 2010. Version 4.10. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

Bird CL. 1954. The dyeing of acetate rayon with disperse dyes. I and II. J Soc Dyers Colourists 70(2):68–77.

Biswas SJ, Khuda-Bukhsh AR. 2005. Cytotoxic and genotoxic effects of the azo-dye *p*-dimethylaminoazobenzene in mice: a time-course study. Mutat Res 587:1–8.

Bonser GM, Clayson DB, Jull JW. 1954. Induction of tumours with 1-(2-tolylazo)-2-naphthol (Oil Orange TX). Nature 174(4436):879–880.

Bonser GM, Clayson DB, Jull JW. 1956. The induction of tumours of the subcutaneous tissues, liver and intestine in the mouse by certain dye-stuffs and their intermediates. Br J Cancer 10:653–667.

Boyland E, Busby ER, Dukes CE, Grover PL, Manson D. 1964. Further experiments on implantation of materials into the urinary bladder of mice. Br J Cancer 13:575–581.

Brambilla B, Carlo P, Finollo R, Ledda A. 1985. Viscometric detection of liver DNA fragmentation in rats treated with ten aromatic amines. Discrepancies with results provided by the alkaline elution technique. Carcinogenesis 6:1285–1288.

Bray HG, Clowes RC, Thorpe WV. 1951. The metabolism of azobenzene and *p*-hydroxyazobenzene in the rabbit. Biochem J 49:lxv.

[BRI] Biopharmaceutical Research Inc. 2011. Testing the metabolism potential of selected aromatic azo and benzidine dyes *in vitro* in two representative human skin bacterial cultures under aerobic conditions. Unpublished report prepared for Health Canada. Vancouver (BC): BRI. Study No. HEA-2011-002.

[BRI] Biopharmaceutical Research Inc. 2012. Testing the metabolism potential of selected aromatic azo and benzidine dyes in vitro in two representative human skin bacterial cultures under aerobic conditions. Unpublished report prepared for Health Canada. Vancouver (BC): BRI. Study No. HEA-2011-002.

[BRI] Biopharmaceutical Research Inc. 2013a. Determination of the metabolic reduction potential of selected aromatic azo- and benzidine-based substances: *in vitro* anaerobic fecal bacterial cultures of the human gastrointestinal tract. Unpublished report prepared for Health Canada. Vancouver (BC): BRI. Study Nos. HEA-2012-001 and HEA-2012-002.

[BRI] Biopharmaceutical Research Inc. 2013b. Identification of azo reductive cleavage products in metabolic reaction of azo dyes by *Staphylococcus epidermidis* and *Micrococcus luteus* under aerobic conditions. Unpublished report prepared for Health Canada. Vancouver (BC): BRI. Study No. HEA-2013-001.

Briggs GG. 1981. Theoretical and experimental relationships between soil adsorption, octanol-water partition coefficients, water solubilities, bioconcentration factors, and the Parachor. J Agric Food Chem 29:1050–1059.

Brockman HE, de Serres FJ, Ong TM, DeMarini DM, Katz AJ, Griffiths AJ, Stafford RS. 1984. Mutation tests in *Neurospora crassa*: a report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat Res 133:87–134.

Broderius SJ, Kahl MD, Hoglund MD. 1995. Use of joint toxic response to define the primary mode of toxic action for diverse industrial organic chemicals. Environ Toxicol Chem 14(9):1591–1605.

Brooks AL, Seiler FA, Hanson RL, Henderson RF. 1989. *In vitro* genotoxicity of dyes present in colored smoke munitions. Environ Mol Mutagen 13:304–313.

Brouns RE, Poot M, De Vrind R, Hoek-Kon TV, Henderson PT. 1979. Measurement of DNA-excision repair in suspensions of freshly isolated rat hepatocytes after exposure to some carcinogenic compounds. Its possible use in carcinogenicity screening. Mutat Res 64:425–432.

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

[BUA] Beratergremium für Umweltrelevante Altstoffe [German Chemical Society Advisory Committee on Existing Chemicals of Environmental Relevance]. 2000. 4-Aminoazobenzene. Stuttgart (DE): S. Hirzel, Wissenschaftliche Verlagsgesellschaft Stuttgart.azo BUA Report No.: 217. Available from: www.hirzel.de/fachbuch/naturwissenschaften/chemie-und-biologie/bua-reports/view/titel/51238.html

Buckingham J. 1982. Dictionary of organic compounds. Volume 5. Ann Arbor (MI): Chapman and Hall Publishers.

Bull RJ, Robinson M, Laurie RD. 1986. Association of carcinoma yield with early papilloma development in SENCAR mice. Environ Health Perspect 68:11–17.

Bursey JT, Pellizzari ED. 1982. Analysis of industrial wastewater for organic pollutants in consent decree survey. Contract No. 68-03-2867. Athens (GA): U.S. Environmental Protection Agency, Environmental Research Lab. pp. 79, 90, 115.

Busk L, Albanus L. 1978. On the mutagenicity of some azo-dyes. Mutat Res 53:161–162.

Caballero F, Meiss R, Gimenez A, Batlle A, Vazquez E. 2004. Immunohistochemical analysis of heme oxygenase-1 in preneoplastic and neoplastic lesions during chemical hepatocarcinogenesis. Int J Exp Pathol 85:213–222.

Cadby PA, Troy WR, Vey MGH. 2002. Consumer exposure to fragrance ingredients: providing estimates for safety evaluation. Regul Toxicol Pharmacol 36:246–252.

[CAMEO Chemicals] Computer-Aided Management of Emergency Operations – Chemicals. Database of Hazardous Materials [database on the Internet]. ©2011. Version 2.4. Washington [DC]: National Oceanic and Atmospheric Administration Service, NOAA's Ocean Service, Office of Response and Restoration. [cited Sept 2011]. Available from: http://cameochemicals.noaa.gov/

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

Campanelli AR, Ferro D, Pavel NV. 1985. Study of the 4:1 inclusion compound between deoxycholic acid and (E)-p-dimethylaminoazobenzene by vapour pressure measurements. Thermochimica Acta 87 :231–238.

Canada. [1978]. *Food and Drug Regulations*, C.R.C., c. 870. Available from: www.canlii.org/en/ca/laws/regu/crc-c-870/latest/crc-c-870.html

Canada. 1999. *Canadian Environmental Protection Act, 1999*. S.C., 1999, c. 33. Canada Gazette, Part III, vol. 22, no. 3. Available from: http://laws-lois.justice.gc.ca/PDF/C-15.31.pdf

Canada. 2000. *Canadian Environmental Protection Act, 1999: Persistence and Bioaccumulation Regulations*, P.C. 2000-348, 29 March 2000, SOR/2000-107. Canada Gazette, Part II, vol. 134, no. 7, p. 607–612. Available from: http://laws-lois.justice.gc.ca/eng/regulations/SOR-2000-107/index.html

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

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

Canada, Dept. of the Environment. 2008b. *Canadian Environmental Protection Act, 1999*: *Notice with respect to Batch 7 Challenge substances.* Canada Gazette, Part I, vol. 142, no. 35, p. 2501–2517. Available from: www.gazette.gc.ca/rp-pr/p1/2008/2008-08-30/pdf/g1-14235.pdf

Canada, Dept. of the Environment. 2009. *Canadian Environmental Protection Act, 1999*: *Notice with respect to certain inanimate substances (chemicals) on the Domestic Substances List*. Canada Gazette, vol. 143, no. 40, p. 2945–2969. Available from: www.gazette.gc.ca/rp-pr/p1/2009/2009-10-03/pdf/g1-14340.pdf

Canada, Dept. of the Environment. 2011. *Canadian Environmental Protection Act, 1999*: *Notice with respect to certain aromatic amines and aromatic azo- and benzidine-based substances.* Canada Gazette, Part I, vol. 145, no. 51, supplement. Available from: www.gazette.gc.ca/rp-pr/p1/2011/2011-12-17/pdf/g1-14551.pdf

Castelain PY. 1967. [Multiple episodes of eczema of the hands caused by sensitization to amino-azotoluene]. 74:561. [in French].

CATALOGIC [Computer Model]. 2012. Version 5.11.6. Bourgas (BG): Bourgas Prof. Assen Zlatarov University, Laboratory of Mathematical Chemistry. Available from: www.oasis-lmc.org/?section=software&swid=1

[CFIA] Canadian Food Inspection Agency. 2010. Food safety action plan report. 2009–2010 Targeted surveys chemistry. Food colours used in the production of manufactured foods. Ottawa (ON): CFIA. Report No.: TS-CHEM-09/10-05.

[CFIA] Canadian Food Inspection Agency. 2011. Food Safety Action Plan report. 2010–2011 targeted surveys chemistry. Food colours used in the production of manufactured foods. Ottawa (ON): CFIA. Report No.: TS-CHEM-10/11.

ChemicalBook [database on the Internet]. 2008. Substance information. [cited January 2013]. Available from: http://www.chemicalbook.com/CASDetailList_1600_EN.htm

ChemNet [database on the Internet]. 2013. Chemical CAS database with Global Chemical Suppliers: CAS 2653-64-7. Available from: http://www.chemnet.com/cas/en/2653-64-7/Solvent%20Red%204.html

Chen et al. 2009. Decolorization of water and oil-soluble azo dyes by *Lactobacillus acidophilus* and *Lactobacillus fermentum*. J Ind Microbiol Biotechnol 36:1459–1466.

Cheung YL, Puddicombe SM, Gray TJB, Ioannides C. 1994. Mutagenicity and CYP1A induction by azobenzenes correlates with their carcinogenicity. Carcinogenesis 15:1257–1263.

Childs JJ, Clayson DB. 1966. The metabolism of 1-phenylazo-2-naphthol in the rabbit. Biochem Pharmacol 15:1247–1258.

Childs JJ, Nakajima C, Clayson DB. 1967. The metabolism of 1-phenylazo-2-naphthol in the rat with reference to the action of the intestinal flora. Biochem Pharmacol 16:1555–1561.

Chiu CW, Lee LH, Wang CY, Bryan GT. 1978. Mutagenicity of some commercially available nitro compounds for *Salmonella typhimurium*. Mutat Res 58:11–22.

[CHRIP] Chemical Risk Information Platform [database on the Internet]. ©2008. Tokyo (JP): National Institute of Technology and Evaluation, Chemical Management Centre (CMC). [cited September 2012]. Available from: www.safe.nite.go.jp/english/db.html

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

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

Clayson DB, Jull JW, Bonser GM. 1958. The testing of ortho hydroxy-amines and related compounds by bladder implantation and a discussion of their structural requirements for carcinogenic activity. Br J Cancer 12:222–230.

Clayson DB, Pringle JA, Bonser GM, Wood M. 1968. The technique of bladder implantation: further results and an assessment. Br J Cancer 22:825–832.

Coles B, Srai SK, Waynforth HB, Ketterer B. 1983. The major role of glutathione in the metabolism and excretion of *N*,*N*-dimethyl-4-aminoazobenzene in the rat. Chem Biol Interact 47:307–323.

Collier SW, Storm JE, Sakr A, Lichtin JL, Bronaugh RL. 1988. The percutaneous absorption and metabolism of azo colors. [Annual Meeting of the Society of Cosmetic Chemists, New York, NY, December 1–2, 1988]. J Soc Cosmet Chem 39:324 (abstract).

Collier SW, Storm JE, Bronaugh RL. 1993. Reduction of azo dyes during *in vitro* percutaneous absorption. Toxicol Appl Pharmacol 118:73–79.

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

Commoner B. 1976. Reliability of bacterial mutagenesis techniques to distinguish carcinogenic and noncarcinogenic chemicals. Washington (DC): US Environmental Protection Agency. Report No.: EPA-600/1-76-022. Available from: http://nepis.epa.gov/Exe/ZyNET.exe/2000Y50K.PDF?ZyActionP=PDF&Client=EPA&Index=1976%20Thru%201980&File=D%3A\ZYFILES\INDEX%20DATA\76THRU80\TXT\00000007\2000Y50K.txt&Query=600176022&SearchMethod=1&FuzzyDegree=0&User=ANONYMOUS&Password=anonymous&QField=pubnum

Commoner B, Vithayathil AJ, Henry JI. 1974. Detection of metabolic carcinogen intermediates in urine of carcinogen-fed rats by means of bacterial mutagenesis. Nature 249:850–852.

Conde-Salazar L, Gonzalez M, Guimaraens D, Meza B. 1991. Allergy contact dermatitis from newspaper ink. Am J Contact Dermatitis 2:245–246.

[ConsExpo] Consumer Exposure Model [Internet]. 2006. Version 4.1. Bilthoven (NL): Rijksinstituut voor Volksgezondheid en Milieu (National Institute for Public Health and the Environment). Available from: www.rivm.nl/en/healthanddisease/productsafety/ConsExpo.jsp#tcm:13-42840

[CPOPs] Canadian Persistent Organic Pollutants Profiler Model. 2012. Version 1.1.18. Gatineau (QC): Environment Canada, Existing Substances Division; Bourgas (BG): Bourgas Prof. Assen Zlatarov University, Laboratory of Mathematical Chemistry. [Model developed based on Mekenyan et al. 2005].

Daniel JW. 1962. The excretion and metabolism of edible food colors. Toxicol Appl Pharmacol 4:572–594.

[Danish EPA] Danish Environmental Protection Agency. 1998. Azocolorants in textiles and toys. Denmark: DTI Clothing and Textiles, Danish Toxicology Centre, VKI Institute for the Water Environment. Report No.: 416 1998.

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

[Danish EPA] Danish Environmental Protection Agency. 2012b. The Danish EPA's risk assessment of hazardous substances in tattoo inks based on the project, "Chemical substances in tattoo ink" [Internet]. Copenhagen (DK): Danish EPA. Available from: www.mst.dk/NR/rdonlyres/10A88C86-7653-4383-840B-F50FD38363C9/0/TheDanishEPARAoftatooinks.pdf

Danneberg P, Schmähl D. 1952. [Estrous-inhibiting substances]. Z Naturforsch 7:468–475. [in German].

Degawa M, Shoji Y, Masuko K, Hashimoto Y. 1979. Mutagenicity of metabolites of carcinogenic aminoazo dyes. Cancer Lett 8:71–76.

Degawa M, Kanazawa C, Hashimoto Y. 1982. *In vitro* metabolism of *o*-aminoazotoluene and mutagenesis of *Salmonella* by the metabolites. Carcinogenesis 3:1113–1117.

Delclos KB, Tarpley WG, Miller EC, Miller JA. 1984. 4-Aminoazobenzene and *N*,*N*-dimethyl-4-aminoazobenzene as equipotent hepatic carcinogens in male C57BL/6 × C3H/He F_1 mice and characterization of *N*-(deoxyguanosin-8-yl)-4-aminoazobenzene as the major persistent hepatic DNA-bound dye in these mice. Cancer Res 44:2540–2550.

Delclos KB, Miller EC, Miller JA, Liem A. 1986. Sulfuric acid esters as major ultimate electrophilic and hepatocarcinogenic metabolites of 4-aminoazobenzene and its *N*-methyl derivatives in infant male C57BL/6J × C3H/HeJ F_1 (B6C3F1) mice. Carcinogenesis 7:277–287.

De Long MJ, Prochaska HJ, Talalay P. 1986. Induction of NAD(P)H:quinone reductase in murine hepatoma cells by phenolic antioxidants, azo dyes, and other chemoprotectors: a model system for the study of anticarcinogens. Proc Natl Acad Sci USA 83:787–791.

De Long MJ, Santamaria AB, Talalay P. 1987. Role of cytochrome P1-450 in the induction of NAD(P)H:quinone reductase in a murine hepatoma cell line and its mutants. Carcinogenesis 8:1549–1553.

Demerec M. 1949. Chemical mutagens. Hereditas 35(Suppl 1):201–209.

Devos SA, van der Valk PG. 2001. The risk of active sensitization to PPD. Contact Dermatitis 44:273–275.

Dimitrov S, Dimitrova N, Walker J, Veith G, Mekenyan O. 2002. Predicting bioconcentration potential of highly hydrophobic chemicals. Effect of molecular size. Pure Appl Chem 74(10):1823–1830.

Dimitrov S, Dimitrova N, Parkerton T, Comber M, Bonnell M, Mekenyan O. 2005. Base-line model for identifying the bioaccumulation potential of chemicals. SAR QSAR Environ Res 16(6):531–554.

DiPaolo JA, Nelson RL, Donovan PJ, Evans CH. 1973. Host-mediated *in vivo–in vitro* assay for chemical carcinogenesis. Arch Pathol 95:380–385.

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

Dračínský M, Cvačka J, Semanská M, Martínek V, Frei E, Stiborová M. 2009. Mechanism of formation of (deoxy)guanosine adducts derived from peroxidase-catalyzed oxidation of the carcinogenic nonaminoazo dye 1-phenylazo-2-hydroxynaphthalene (Sudan I). Chem Res Toxicol 22:1765–1773.

Dragan YP, Rizvi T, Xu YH, Hully JR, Bawa N, Campbell HA, Maronpot RR, Pitot HC. 1991. An initiation–promotion assay in rat liver as a potential complement to the 2-year carcinogenesis bioassay. Fundam Appl Toxicol 16:525–547.

Droste R. 1997. Theory and practice of water and wastewater treatment. New York (NY): John Wiley & Sons.

Druckrey H. 1967. Quantitative aspects in chemical carcinogenesis. In: Truhaut R, editor. Potential carcinogenic hazards from drugs. (UICC Monograph Series, vol. 7). Berlin (DE): Springer; p. 60–78.

Druckrey H, Küpfmüller K. 1948. Quantitative analyse der krebsentstehung. Z Naturforsch 3(b):254–266.

[DS TOPKAT] Discovery Studio TOxicity Prediction by Komputer Assisted Technology [Prediction Module]. ©2005–2009. Version 2.5.0.9164. San Diego (CA): Accelrys Software Inc. Available from: www.accelrys.com/products/

Dunkel VC, Pienta RJ, Sivak A, Traul KA. 1981. Comparative neoplastic transformation responses of Balb/3T3 cells, Syrian hamster embryo cells, and Rauscher murine leukemia virus-infected Fischer 344 rat embryo cells to chemical compounds. J Natl Cancer Inst 67:1303–1315.

Dunkel VC, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K, Rosenkranz HS, Simmon VF. 1984. Reproducibility of microbial mutagenicity assays: i. Tests with *Salmonella typhimurium* and *Escherichia coli* using a standardized protocol. Environ Mol Mutagen 6(Suppl 2):1–254.

[ECHA] European Chemicals Agency. ©2007–2013. Registered substances database. Helsinki (FI): European Chemicals Agency. [updated 2012 Nov27; cited March 2013]. Available from: www.echa.europa.eu/information-on-chemicals/registered-substances

[ECHA] European Chemicals Agency. 2008. Guidance on information requirements and chemical safety assessment. Chapter R.11: PBT assessment. May 2008. Guidance for the implementation of REACH. Helsinki (FI): European Chemicals Agency.

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

[ECJRC] European Commission Joint Research Centre. European Union risk assessment report: CAS: 90-04-0: *o*-anisidine [Internet]. 2002. Luxembourg: Office for Official Publications of the European Communities. Report No.: EUR 19834 EN. Available from: http://esis.jrc.ec.europa.eu/doc/risk_assessment/REPORT/o-anisidinereport025.pdf

[ECJRC] European Commission Joint Research Centre. 2003. Technical guidance document on risk assessment: Part II. European Chemicals Bureau, Institute for Health and Consumer Protection. Luxembourg: Office for Official Publications of the European Communities. Report No.: EUR 20418 EN/2.

Edwards CN, Combes RD. 1981. An evaluation of the *Drosophila zeste* somatic eye mutation test for the detection of genotoxic azo dyes and an aromatic amine. Mutat Res 84:221–226.

[EFSA] European Food Safety Authority. 2005. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission to review the toxicology of a number of dyes illegally present in food in the EU. EFSA J 263:1–71.

Elliott BM, Griffiths K, Mackay JM, Wade JD. 1997. CI Solvent Yellow 14 shows activity in the bone marrow micronucleus assay in both the rat and mouse. Mutagenesis 12:255–258.

Elmore E, Fitzgerald MP. 1990. Evaluation of the bioluminescence assays as screens for genotoxic chemicals. Prog Clin Biol Res 340D:379–387.

Elson LA, Warren FL. 1944. The metabolism of azo compounds: 1. Azobenzene. Biochem J 38:217–220.

Environment Canada. 2006. Data for selected substances collected under the *Canadian Environmental Protection Act, 1999*, section 71: *Notice with respect to selected substances identified as priority for action.* Data prepared by: Environment Canada, Existing Substances Program.

Environment Canada. 2008. Data for Batch 6 substances collected under the *Canadian Environmental Protection Act, 1999*, section 71: *Notice with respect to certain Batch 6 Challenge substances.* Data prepared by: Environment Canada, Existing Substances Program.

Environment Canada. 2009. Data relating to the Domestic Substances List (DSL) Inventory Update 2008 collected under the *Canadian Environmental Protection Act, 1999*, section 71: *Notice with respect to certain inanimate substances (chemicals) on the Domestic Substances List*. Data prepared by: Environment Canada, Existing Substances Program.

Environment Canada. 2013. Internal database of Canadian surface water sediments. Ottawa (ON). Ecological Assessment Division, Science and Risk Assessment Directorate, Environment Canada.

Environment Canada. 2014. Supporting documentation for the Screening Assessment of Aromatic Azo and Benzidine-based Substance Grouping: Certain Azo Solvent Dyes (March 2015). Gatineau (QC): Environment Canada. [last updated 2014-07-10]. Available on request from: substances@ec.gc.ca

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

Environment Canada, Health Canada. 2008. Final screening assessment report for the screening assessment of 145 PBiT substances. [Internet]. Ottawa (ON): Environment Canada, Health Canada. [cited 2014 July]. Available from: http://www.chemicalsubstanceschimiques.gc.ca/plan/approach-approche/pbit145-eng.php#a3

Environment Canada, Health Canada. 2009. Screening Assessment for the Challenge: 2-Naphthalenol, 1-[(2-methoxyphenyl)azo]- (Solvent Red 1), Chemical Abstracts Service Registry Number 1229-55-6; 2-Naphthalenol, 1-[(2,4-dimethylphenyl)azo]- (Solvent Orange 7), Chemical Abstracts Service Registry

Number 3118-97-6; 1-Naphthalenol, 4-[(4-ethoxyphenyl)azo]- (Solvent Red 3), Chemical Abstracts Service Registry Number 6535-42-8 [Internet]. Ottawa (ON): Environment Canada, Health Canada. [cited 2012 Sep]. Available from: www.ec.gc.ca/ese-ees/E5E72B73-2E7C-4E39-91F0-32B2C1CC8E7F/batch6_3118-97-6_en.pdf

Environment Canada, Health Canada. 2010. Screening Assessment for the Challenge: 3H-Pyrazol-3-one, 4-[(2-chlorophenyl)azo]-2,4-dihydro-5-methyl-2-phenyl- (Pigment Yellow 60), Chemical Abstracts Service Registry Number 6407-74-5; 3H-Pyrazol-3-one, 4-[(2,4-dimethylphenyl)azo]-2,4-dihydro-5-methyl-2-phenyl- (Solvent Yellow 18), Chemical Abstracts Service Registry Number 6407-78-9; [1,1′-Biphenyl]-4,4′-diamine, *N*,*N*′-bis(2,4-dinitrophenyl)-3,3′-dimethoxy- (Pigment Brown 22), Chemical Abstracts Service Registry Number 29398-96-7; 1-Naphthalenemethanol, α,α-bis[4-(diethylamino)phenyl]-4-(ethylamino)- (Solvent Blue 5), Chemical Abstracts Service Registry Number 1325-86-6; 1-Naphthalenemethanol, α,α-bis[4-(dimethylamino)phenyl]-4-(phenylamino)- (Solvent Blue 4), Chemical Abstracts Service Registry Number 6786-83-0 [Internet]. Ottawa (ON): Environment Canada, Health Canada. [cited 2012 Sep]. Available from: www.ec.gc.ca/ese-ees/default.asp?lang=En&xml=95100726-A2FF-FEB1-0073-A5406988234D

Environment Canada, Health Canada. 2011. Screening Assessment for the Challenge: 2-Naphthalenol, 1-[[4-(phenylazo)phenyl]azo]- (Solvent Red 23), Chemical Abstracts Service Registry Number 85-86-9 [Internet]. Ottawa (ON): Environment Canada, Health Canada. [cited 2012 Sep]. Available from: www.ec.gc.ca/ese-ees/5B828E08-3266-4189-B32B-39B5D773040A/Batch%206_85-86-9_EN.pdf

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

Environment Canada, Health Canada. 2013b. Draft Screening Assessment: Aromatic Azo and Benzidine-based Substance Grouping. Certain Azo Disperse Dyes. Ottawa (ON): Environment Canada, Health Canada. Available from: http://www.chemicalsubstanceschimiques.gc.ca/group/index-eng.php

Environment Canada, Health Canada. 2014a. Draft Screening assessment: Aromatic Azo and Benzidine-based Substance Grouping. Certain Azo Basic Dyes. Ottawa (ON): Environment Canada, Health Canada.

Environment Canada, Health Canada. 2014b. Draft Screening Assessment: Aromatic Azo and Benzidine-based Substance Grouping. Certain Aromatic Amines. Ottawa (ON): Environment Canada, Health Canada.

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

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

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

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

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

[ETAD] Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers. 1997. Extractability of dyestuffs from textiles over a normal life time of use. Basel (CH): ETAD. Project G 1033.

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

European Commission. ©2000. IUCLID Dataset, *p*-Phenetidine, CAS No. 156-43-4 [Internet]. Year 2000 CD-ROM edition. [place unknown]: European Chemicals Agency, European Commission. [created 2000 Feb 18; cited yr mon date]. Available from: http://esis.jrc.ec.europa.eu/doc/IUCLID/data_sheets/156434.pdf

European Commission. 2008. Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006. Official Journal of the European Union L 353:1–1355. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:353:0001:1355:en:PDF

Fare G. 1966. Rat skin carcinogenesis by topical applications of some azo dyes. Cancer Res 26:2406–2408.

Fassina G, Abbondandolo A, Mariani L, Taningher M, Parodi S. 1990. Mutagenicity in V79 cells does not correlate with carcinogenity in small rodents for 12 aromatic amines. J Toxicol Environ Health 29:109–130.

Feldmann RJ, Maibach HI. 1970. Absorption of some organic compounds through the skin in man. J Invest Dermatol 54(5):399–404.

Fischer W. 1954. Durch Buttergelb erzeugte Tumoren. Arch Geschwulstforch 7:301–320.

Fitzhugh OG, Nelson AA, Bourke R 1956. Chronic toxicities of two food colors, FD & C Red No. 32 and FD & C Orange No. 2. Fed Proc 13:422.

Fochtman EG. 1981. Biodegradation and carbon adsorption of carcinogenic and hazardous compounds. Project Summary. Cincinnati (OH): U.S. Environmental Protection Agency, Research and Development, Municipal Environmental Research Laboratory, EPA-600/S2-81-032. March 1981.

Food Standards Agency. 2005. Summary report of LA activity and key findings from imported food sampling and surveillance grants 2004/05. July 2005, Version 1.1 [Internet]. London (GB): United Kingdom Food Standards Agency. Available from: www.food.gov.uk/multimedia/pdfs/impsamp200405.pdf

Foureman P, Mason JM, Valencia R, Zimmering S. 1994. Chemical mutagenesis testing in *Drosophila*. X. Results of 70 coded chemicals tested for the National Toxicology Program. Environ Mol Mutagen 23:208–227.

Foussereau J. 1985. An allergen in a judo club? Contact Dermatitis 13:283.

Foussereau J, Escande JP, Lantz JP, Grosshans E, Wick P. 1973. Sensitisation to *ortho*-aminoazotoluene. Trans St John's Hosp Dermatol Soc 59:251–260.

Fouts JR, Kamm JJ, Brodie BB. 1957. Enzymatic reduction of prontosil and other azo dyes. J Pharmacol Exp Ther 120:291–300.

Francalanci S, Giorgini S, Ricci L, Sertoli A. 2001. Patch testing by additional series of allergens: results of further experiences. Am J Contact Dermatitis 12:203–207.

Freeman AE, Weisburger EK, Weisburger JH, Wolford RG, Maryak JM, Huebner RJ. 1973. Transformation of cell cultures as an indication of the carcinogenic potential of chemicals. J Natl Cancer Inst 51:799–808.

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

French JE, Saulnier M. 2000. Benzene leukemogenesis: an environmental carcinogen-induced tissue-specific model of neoplasia using genetically altered mouse models. J Toxicol Environ Health A 61(5-6):377-9.

Fujii K. 1983. Induction of tumors in transplacental or neonatal mice administered 3′-methyl-4-dimethylaminoazobenzene or 4-aminoazobenzene. Cancer Lett 17:321–325.

Fujimoto K, Hashimoto S, Kozuka T, Tashiro M, Sano S. 1985. Occupational pigmented contact dermatitis from azo-dyes. Contact Dermatitis 12:15–17.

Fujita S, Adachi S, Uesugi T. 1982. Effect of 1-*m*-tolueneazo-2-naphthol on hepatic drug metabolism. I. Induction of cytochrome P-448. J Pharmacobiodyn 5:259–265.

Fujita S, Suzuki M, Peisach J, Suzuki T. 1984. Induction of hepatic microsomal drug metabolism by azo compounds: a structure–activity relationship. Chem Biol Interact 52:15–38.

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

Gatehouse DG, George E, Westmoreland C. 1991. Investigations into the adequacy of two *in vivo* genotoxicity assays (mouse micronucleus test, rat liver UDS assay) for the detection of genotoxic carcinogens. [Twenty-second Annual Scientific Meeting of the Environmental Mutagen Society, Kissimmee, FL, April 6–11, 1991]. Environ Mol Mutagen 17(Suppl 19):81 (abstract).

Gaudin D, Gregg RS, Yielding KL. 1971. DNA repair inhibition. Possible mechanism of action of cocarcinogens. Biochem Biophys Res Commun 45:630–636.

Geier J, Lessmann H, Dickel H, Frosch PJ, Koch P, Becker D, Jappe U, Aberer W, Schnuch A, Uter W. 2004. Patch test results with the metalworking fluid series of the German Contact Dermatitis Research Group (DKG). Contact Dermatitis 51:118–130.

Gelstein VI. 1961. Incidence of tumours in descendants of mice treated with orthoaminoazotoluene. Vop Onkol 7:58–64. [cited in IARC 1975b].

George E, Andrews M, Westmoreland C. 1990. Effects of azobenzene and aniline in the rodent bone marrow micronucleus test. Carcinogenesis 11:1551–1555.

Gershbein LL. 1982. Action of dyes and indicators on rat-liver regeneration. Food Chem Toxicol 20:1–8.

Giusti F, Seidenari S. 2003. Textile dyes sensitization: a study of 49 patients allergic to disperse dye alone. Contact Dermatits 48:54–55.

Giusti F, Mantovani L, Martella A, Seidenari S. 2002. Hand dermatitis as an unsuspected presentation of textile dye contact sensitivity. Contact Dermatitis 47:91–95.

Giusti F, Massone F, Bertoni L, Pellacani G, Seidenari S. 2003. Contact sensitization to disperse dyes in children. Pediatr Dermatol 20:393–397.

Gobas F. 2007. Development and review of a generic water–sediment modelling framework for organic chemicals. Report prepared for Environment Canada. Burnaby (BC): Simon Fraser University, Faculty of Environment. March 26, 2007.

Gobas F. 2010. Comments on approach to sediment exposure approach. Report prepared for Environment Canada. Burnaby (BC): Simon Fraser University, Faculty of Environment. March 25, 2010.

Goh CL, Kozuka T. 1986. Pigmented contact dermatitis from “kumkum.” Clin Exp Dermatol 11:603–606.

Golka K, Kopps S, Prager HM, Mende SV, Thiel R, Jungmann O, Zumbe J, Bolt HM, Blaszkewicz M, Hengstler JG, Selinski S. 2012. Bladder cancer in crack testers applying azo dye-based sprays to metal bodies. J Toxicol Environ Health 75:566–571.

Goodman DG, Ward JM, Reichardt WD. 1984. Splenic fibrosis and sarcomas in F344 rats fed diets containing aniline hydrochloride, *p*-chloroaniline, azobenzene, *o*-toluidine hydrochloride, 4,4′-sulfonyldianiline, or D & C Red No. 9. J Natl Cancer Inst 73:265–273.

Gosselin RE, Smith RP, Hodge HC, Braddock JE, editors. 1984. Clinical toxicology of commercial products. 5th ed. Baltimore (MD): Williams & Wilkins; p. II–289. [cited in HSDB 1983–].

Green FJ. 1990. The Sigma-Aldrich handbook for dyes, stains and indicators. Milwaukee (WI): Aldrich Chemical Co. p. 513. As cited in PhysProp (2006).

Green HS, Jones F. 1967. Vapour pressures, heats of sublimation and degree of association of some azo compounds in the vapour phase. Trans Faraday Soc 63:1612–1619.

Guidechem [database on the Internet]. ©2010–2013. Chemical Trading Guide. [cited 2013 Jan]. Available from: http://www.guidechem.com/

Hackmann C. 1951. Carcinogenic effect of some fat-soluble azo dyes. Z Krebsforsch 57:530–541.

Hakura A, Shimada H, Nakajima M, Sui H, Kitamoto S, Suzuki S, Satoh T. 2005. *Salmonella*/human S9 mutagenicity test: a collaborative study with 58 compounds. Mutagenesis 20:217–228.

Hansch C, Leo A, Hoekman D. 1995. Exploring QSAR: Hydrophobic, electronic, and steric constants. Washington (DC): American Chemical Society, ACS Professional Reference Book. As cited in EPI Suite 2012.

Hariya T, Hatao M, Ichikawa H. 1999. Development of a non-radioactive endpoint in a modified local lymph node assay. Food Chem Toxicol 37:87–93.

Harrington-Brock K, Parker L, Doerr C, Cimino MC, Moore MM. 1991. Analysis of the genotoxicity of anthraquinone dyes in the mouse lymphoma assay. Mutagenesis 6:35–46.

Hart RW, Freni SC, Gaylor DW, Gillette JR, Lowry LK, Ward JM, Weisburger EK, Lepore P, Turturro A. 1986. Final report of the Color Additive Scientific Review Panel. Risk Anal 6:117–154.

Hashimoto Y, Watanabe HK, Degawa M. 1981. Mutagenicity of 4-aminoazobenzene, *N*-hydroxy-4-aminoazobenzene, 4-nitrosoazobenzene, 4-nitroazobenzene, and their ring methoxylated derivatives on *Salmonella*. Gann 72:921–929-9.

Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E. 1983. *Salmonella* mutagenicity test results for 250 chemicals. Environ Mutagen 5(Suppl 1):3–142.
Health Canada. 1994. Human health risk assessment for priority substances. Ottawa (ON): Health Canada. Available from: www.hc-sc.gc.ca/ewh-semt/alt_formats/hecs-sesc/pdf/pubs/contaminants/approach/approach-eng.pdf

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

Health Canada. 2011. *Canadian Environmental Protection Act, 1999*: Follow-up report on a PSL substance for aniline (CASRN 62-53-3). Ottawa (ON): Health Canada. Available from: www.ec.gc.ca/ese-ees/CCDA73CC-13BC-4835-AA8D-966C159B56F2/Aniline_Follow-up_EN.pdf

Health Canada. 2012. List of Permitted Colouring Agents (Lists of Permitted Food Additives). Ottawa (ON): Health Canada. [cited 2013 Feb]. Available from: www.hc-sc.gc.ca/fn-an/securit/addit/list/3-colour-color-eng.php

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

Health Canada. 2014. The Cosmetic Ingredient Hotlist 2014 [Internet]. Ottawa (ON): Health Canada, Consumer Product Safety. [cited 2014 Sept]. Available from: http://www.hc-sc.gc.ca/cps-spc/alt_formats/pdf/cosmet-person/hot-list-critique/hotlist-liste-eng.pdf

Heidelberger C, Freeman AE, Pienta RJ. 1983. Cell transformation by chemical agents—a review and analysis of the literature. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat Res 114:283–385.

Hellmér L, Bolcsfoldi G. 1992a. An evaluation of the *E. coli* K-12 uvrB/recA DNA repair host-mediated assay. I. *In vitro* sensitivity of the bacteria to 61 compounds. Mutat Res 272:145–160.

Hellmér L, Bolcsfoldi G. 1992b. An evaluation of the *E. coli* K-12 uvrB/recA DNA repair host-mediated assay. II. *In vivo* results for 36 compounds tested in the mouse. Mutat Res 272:161–173.

Henderson RF, Brooks AL, Hanson RL. 1986. Genotoxicity of dyes present in colored smoke munitions. Albuquerque (NM): Lovelace Biomedical & Environmental Research Institute, Inc.

Henderson RF, Bechtold WE, Medinsky MA, Fischer JP, Lee TT. 1988. The effect of molecular weight/lipophilicity on clearance of organic compounds from lungs. Toxicol Appl Pharmacol 95:515–521.

Henry MC. 1983. Mutagenic screening of six candidate dyes for colored smoke munitions in the *Salmonella* reversion assay. Fort Detrick (MD): US Army Medical Bioengineering Research and Development Laboratory.

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

Heukelekian H, Rand MC. 1955. Biological oxygen demand of pure organic chemicals: a report of the Research Committee, FSIWA. Water Environment Federation. Sewage and Industrial Wastes 27(9):1040–1053.

Hillen U, Grabbe S, Uter W. 2007. Patch test results in patients with scalp dermatitis: analysis of data of the Information Network of Departments of Dermatology. Contact Dermatitis 56:87–93.

Holcombe GW, Phipps GL, Knuth ML, Felhaber T. 1984. The acute toxicity of selected substituted phenols, benzenes and benzoic acid esters to fathead minnows *Pimephales promelas*. Environ Pollut Ser A 35(4):367–381.

Holden CR, Gawkrodger DJ. 2005. 10 years' experience of patch testing with a shoe series in 230 patients: which allergens are important? Contact Dermatitis 53:37–39.

Hou M, Baughman GL, Perenich TA. 1991. Estimation of water solubility and octanol/water partition coefficient of hydrophobic dyes. Part II: Reverse-phase high performance liquid chromatography. Dyes Pigments 16:291–297.

Household Products Database [database on the Internet]. 1993– . Bethesda (MD): National Library of Medicine (US). [cited 2012 June]. Available from: www.householdproducts.nlm.nih.gov/

[HSDB] Hazardous Substances Data Bank [database on the Internet]. 1983– . Bethesda (MD): National Library of Medicine (US). [revised 2006 Dec 20; cited 2012 and 2013]. Available from: www.toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB

Huang H, Yu Y, Jing L, Wang X, Feng J, Niu H, Xiao Q, Wang L. 2004. Semivolatile Organic Pollutants in Water, Suspended Solids, and Surface Sediments of the Huaihe River, Jiangsu Section, People's Republic of China. Bull Environ Contam Toxicol 73(2):339–346.

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

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1975a. *para*-Aminoazobenzene. IARC Monogr Eval Carcinog Risks Hum 8:53–60.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1975b. *ortho*-Aminoazotoluene. IARC Monogr Eval Carcinog Risks Hum 8:61–74.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1975c. Azobenzene. IARC Monogr Eval Carcinog Risks Hum 8:75–81.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1975d. Chrysoidine. IARC Monogr Eval Carcinog Risks Hum 8:91–96.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1975e. C.I. Disperse Yellow 3. IARC Monogr Eval Carcinog Risks Hum 8:97–100.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1975f. *para*-Dimethylaminoazobenzene. IARC Monogr Eval Carcinog Risks Hum 8:125–146.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1975g. Oil Orange SS. IARC Monogr Eval Carcinog Risks Hum 8:165–171.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1975h. Scarlet Red. IARC Monogr Eval Carcinog Risks Hum 8:217–224.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1975i. Sudan I. IARC Monogr Eval Carcinog Risks Hum 8:225–231.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1975j. Sudan Red 7B. IARC Monogr Eval Carcinog Risks Hum 8:253–256.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1987a. Overall evaluations of carcinogenicity: an updating of IARC Monographs Volumes 1 to 42. IARC Monogr Eval Carcinog Risks Hum Suppl 7. Available from: http://monographs.iarc.fr/ENG/Monographs/suppl7/index.php

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1987b. Chrysoidine (group 3). IARC Monogr Eval Carcinog Risks Hum Suppl 7:169.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 1990. Disperse Yellow 3. IARC Monogr Eval Carcinog Risks Hum 48:149–159.

[IARC] IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. 2012. *ortho*-Toluidine. IARC Monogr Eval Carcinog Risks Human 100F:93–100. Available from: http://monographs.iarc.fr/ENG/Monographs/vol100F/mono100F-11.pdf

Ichinotsubo D, Mower HF, Setliff J, Mandel M. 1977. The use of rec− bacteria for testing of carcinogenic substances. Mutat Res 46:53–62.

Idaka E, Ogawa T. 1978. Degradation of azo compounds by *Aeromonas hydrophíla* var. 24B. J Soc Dyers Colorists 3:91–94.

Ikarashi Y, Tsuchiya T, Nakamura A. 1996. Application of sensitive mouse lymph node assay for detection of contact sensitization capacity of dyes. J Appl Toxicol 16:349–354.

[ILS] Integrated Laboratory Systems, Inc. 2011. Assessment of the mutagenicity of twenty aromatic azo substances in the Ames mutagenicity assay using the Prival modification with and without FMN. Unpublished report. Research Triangle Park (NC): ILS. ILS Project Study No. C191-006.

Imokawa G, Yada Y, Okuda M. 1992. Allergic contact dermatitis releases soluble factors that stimulate melanogenesis through activation of protein kinase C–related signal-transduction pathway. J Invest Dermatol 99:482–488.

Industry Canada. 2011. Trade data online (TDO). Explanatory notes—trade type [Internet]. [cited 2012 Aug 2]. Available from: www.ic.gc.ca/eic/site/tdo-dcd.nsf/eng/h_00012.html

Ishidate M, Hashimoto Y. 1962. Metabolism of 4-dimethylaminoazobenzene and related compounds. II. Metabolites of 4-dimethylaminoazobenzene and 4-aminoazobenzene in rat urine. Chem Pharm Bull (Tokyo) 10:125–133.

Ishidate M Jr, Odashima S. 1977. Chromosome tests with 134 compounds on Chinese hamster cells *in vitro*: a screening for chemical carcinogens. Mutat Res 48:337–354.

Ishikawa Y, Galuser J, Janshekar H. 2008. CEH marketing research report: dyes. Menlo Park (CA): SRI Consulting. Available upon request.

Ito Y, Maeda S, Fujihara T, Ueda N, Sugiyama T. 1982. Suppression of 7,12-dimethylbenz[*a*]anthracene-induced chromosome aberrations in rat bone marrow cells after treatment with Sudan III (Solvent Red 23) and related azo dyes. J Natl Cancer Inst 69:1343–1346.

Ivett JL, Brown BM, Rodgers C, Anderson BE, Resnick MA, Zeiger E. 1989. Chromosomal aberrations and sister chromatid exchange tests in Chinese hamster ovary cells *in vitro*. IV. Results with 15 chemicals. Environ Mol Mutagen 14:165–187.

Jaffe WG. 1947. The response of mice to the simultaneous application of two different carcinogenic agents. Cancer Res 7:529–530.

Janado M, Takenaka K, Nakamori H, Yano Y. 1980. Solubilities of water-insoluble dyes in internal water of swollen sephadex gels. J Biochem 87(1):57–62.

Ji Q, Liu G, Zhou J, Wang J, Jin R, Lv H. 2012. Removal of water-insoluble Sudan dyes by *Shewanella oneidensis* MR-1. Bioresour Technol 114:144–148.

Ji Y, Ji C, Gao S, Yu L. 2006a. [Study on effect of Sudan I, III and IV on proliferation of SGC-7901 by flow cytometry]. Harbin Gongye Daxue Xuebao 38:767–769. [in Chinese].

Ji Y, Ji C, Gao S, Lang L. 2006b. A study of the effect of Sudan I, III, and IV on the DNA/RNA ratio and 3D structure of HepG-2 using LCM. J Harbin Inst Technol (Engl ed) 13:173–177.

Johnson GE, Quick EL, Parry EM, Parry JM. 2010. Metabolic influences for mutation induction curves after exposure to Sudan-1 and Para Red. Mutagenesis 25:327–333.

Jones AH. 1960. Sublimation-pressure data for organic compounds. J Chem Eng Data 5:196-200. As cited in EPI Suite 2012.

Jordan WP Jr, Dahl MV. 1972. Contact dermatitis from cellulose ester plastics. Arch Dermatol 105:880–885.

Jull JW. 1979. The effect of time on the incidence of carcinomas obtained by the implantation of paraffin wax pellets into mouse bladder. Cancer Lett 6:21–25.

Jungclaus GA, Lopez-Avila V, Hites RA. 1978. Organic compounds in an industrial wastewater: a case study of their environmental impact. Environ Sci Technol 12:88–96.

Kada T, Tutikawa K, Sadaie Y. 1972. *In vitro* and host-mediated “rec-assay” procedures for screening chemical mutagens; and phloxine, a mutagenic red dye detected. Mutat Res 16:165–174.

Kada T, Hirano K, Shirasu Y. 1980. Screening of environmental chemical mutagens by the *rec*-assay system with *Bacillus subtilis*. In: Hollaender A, de Serres FJ, editors. Chemical mutagens: principles and methods for their detection, vol. 6. New York (NY): Plenum; p. 149–173.

Kaledin VI, Alekseeva GV, Volkova AI. 1978. Carcinogenicity of orthoaminoazotoluene for mouse intestines. Bull Exp Biol Med 86:476–477.

Karickhoff S. 1981. Semi-empirical estimation of sorption of hydrophobic pollutants on natural sediments and soils. Chemosphere 10:833–846.

Kawachi T, Yahagi T, Kada T, Tazima Y, Ishidate M, Sasaki M, Sugiyama T. 1980a. Cooperative program on short-term assays for carcinogenicity in Japan. IARC Sci Publ 27:323–330.

Kawachi T, Komatsu T, Kada T, Ishidate M, Sasaki M, Sugiyama T, Tazima Y. 1980b. Results of recent studies on the relevance of various short-term screening tests in Japan. In: Williams GM, Kroes R,

Waaijer HW, van de Poll KW, editors. The predictive value of short-term screening tests in carcinogenicity evaluation. Amsterdam (NL): Elsevier.

Kawajiri K, Yonekawa H, Harada N, Noshiro M, Omura T, Tagashira Y. 1980. Immunochemical study on the role of different types of microsomal cytochrome P-450 in mutagenesis by chemical carcinogens. Cancer Res 40:1652–1657.

Kawano S, Kamataki T, Maeda K, Kato R, Nakao R, Mizoguchi I. 1985. Activation and inactivation of a variety of mutagenic compounds by the reconstituted system containing highly purified preparations of cytochrome P-450 from rat liver. Fundam Appl Toxicol 5:487–498.

Khramkova NI, Guelstein VI. 1965. Antigenic structure of mouse hepatomas. V. Organospecific liver antigens and embryonic alpha-globulin in hepatomas of mice induced with orthoaminoazotoluene (AAT). Neoplasma 12:239–250.

Khudoley VV. 1972. Induction of liver tumors by some azo compounds in aquarium guppies [*Lebistes reticulates* (Peters)]. J Ichthyol 12:319–324.

Kim K, Shin H. 2000. Reduction of azobenzene by purified bovine liver quinone reductase. J Biochem Mol Biol 33:321–325.

Kim VKH. 1973. [A study of chromosomal aberrations in the course of radiation and chemical carcinogenesis]. Tsitologiya 15(5):578–584. [in Russian].

Kinosita R. 1936. [Researches on the cancerogenesis of the various chemical substances]. Gann 30:423–426. [in Japanese].

Kinosita R. 1937. Special report. Studies on the cancerogenic chemical substances. Trans Jpn Pathol Soc 21:665–727.

Kirby AHM. 1945a. Studies in carcinogenesis with azo compounds. I. The action of four azo dyes in mixed and pure strain mice. Cancer Res 5:673–682.

Kirby AHM. 1945b. Studies in carcinogenesis with azo compounds. II. The action of azo compounds in mice, and the bearing thereof on theories of azo dye carcinogenesis. Cancer Res 5:683–696.

Kirby AHM. 1947. Studies in carcinogenesis with azo compounds. III. The action of four azo compounds in Wistar rats fed restricted diets; *N*,*N*-diethyl-*p*-aminoazobenzene in mice. Cancer Res 7:333–341.

Kirby AHM, Peacock PR. 1947. The induction of liver tumours by 4-aminoazobenzene and its *N*:*N*-dimethyl derivative in rats on a restricted diet. J Pathol Bacteriol 59:1–7.

Kirkhart B. 1981. Micronucleus test on 21 compounds. Prog Mutat Res 1:698–704.

[Kirk-Othmer] Kirk-Othmer Encyclopedia of Chemical Technology. Online version. 2010. Available from: http://onlinelibrary.wiley.com/book/10.1002/0471238961/ [restricted access]

Kitchin KT, Brown JL. 1994. Dose–response relationship for rat liver DNA damage caused by 49 rodent carcinogens. Toxicology 88:31–49.

Kitchin KT, Brown JL, Kulkarni AP. 1992. Predictive assay for rodent carcinogenicity using *in vivo* biochemical parameters: operational characteristics and complementarity. Mutat Res 266:253–272.

Kitchin KT, Brown JL, Kulkarni AP. 1994. Complementarity of genotoxic and nongenotoxic predictors of rodent carcinogenicity. Teratog Carcinog Mutagen 14:83–100.

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

Kohara A, Suzuki T, Honma M, Hirano N, Ohsawa K, Ohwada T, Hayashi M. 2001. Mutation spectrum of *o*-aminoazotoluene in the *cII* gene of lambda/lacZ transgenic mice (MutaMouse). Mutat Res 491:211–220.

Kondo K, Miyajima H. 1997. Micronucleus test of Solvent Yellow 14 in both peripheral blood and bone marrow of rats and mice. [7th International Conference on Environmental Mutagens, September 7–12, 1997, Toulouse, France]. Mutat Res 379 (1 Suppl 1):S93 (abstract).

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

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

Kornbrust DJ, Barfknecht TR. 1985b. Comparison of azo dyes and their reduction products genotoxicity in rat and hamster hepatocyte DNA repair assays. [16th Annual Meeting of the Environmental Mutagen Society, Las Vegas, NV, February 25 – March 1, 1985]. Environ Mutagen 7(Suppl 3):70 (abstract).

Kornbrust D, Dietz D. 1987. Effects of pretreatment with inducers of hepatic mixed function oxidases on DNA repair elicited by various compounds in hepatocytes from adult and neonatal rats. Cell Biol Toxicol 3:143–164.

Kowalski LA, Assi KP, Wee RK, Madden Z. 2001. *In vitro* prediction of carcinogenicity using a bovine papillomavirus DNA-carrying C3H/10T1/2 cell line (T1). II: Results from the testing of 100 chemicals. Environ Mol Mutagen 37:231–240.

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

Kozuka T, Tashiro M, Fujimoto K, Nakamura Y, Hashimoto S, Nakaminami G. 1979. Skin sensitivity of commercial red 225. Hifu 21(3):293–296.

Kozuka T, Tashiro M, Sano S, Nakamura Y, Hashimoto S, Nakaminami G, Fujimoto K. 1980. Pigmented contact dermatitis from azo dyes: 1. Cross-sensitivity in humans. Contact Dermatitis 6:330–336.

Kühn R, Pattard M, Pernak KD, Winter A. 1988. Schadstoffwirkungen von Umweltchemikalien im Daphnien-Reproduktionstest als Grundlage zur Bewertung der Umweltgefahrlichkeit in aquatischen Systemen. Forschungsbericht: 10603052, Umweltbundesamt, Bismarckplatz I, D-1000 Berlin 33. As cited in Kühn and Pattard (1990).

Kühn R, Pattard M. 1990. Results of the harmful effects of water pollutants to green algae (*Scenedesmus subspicatus*) in the cell multiplication inhibition test. Water Res 24(1):31–38.

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

Kujawa M, Macholz R, Bleyl D, Nickel B, Seidler H. 1985. [Analysis of azoxybenzene and its metabolites as well as the placental transfer of azoxybenzene.] Z Gesamte Hyg Ihre Grenzgeb 31(8):464–465. [in German].

Lake BG, Edwards AJ, Price RJ, Phillips BJ, Renwick AB, Beamand JA, Adams TB. 2001. Lack of effect of furfural on unscheduled DNA synthesis in the *in vivo* rat and mouse hepatocyte DNA repair assays and in precision-cut human liver slices. Food Chem Toxicol 39:999–1011.

Lake RS, Kropko ML, Pezzutti MR, Shoemaker RH, Igel HJ. 1978. Chemical induction of unscheduled DNA synthesis in human skin epithelial cell cultures. Cancer Res 38:2091–2098.

Latt SA, Allen J, Bloom SE, Carrano A, Falke E, Kram D, Schneider E, Schreck R, Tice R, Whitfield B, Wolff S. 1981. Sister-chromatid exchanges: a report of the GENE-TOX program. Mutat Res 87:17–62.

Law LW. 1941. The cancer producing properties of azo compounds in mice. Cancer Res 1:397–401.

Lawson TA. 1970. The effect of prolonged feeding of *ortho*-aminoazotoluene on binding to cellular constituents in mouse liver. Chem Biol Interact 2:9–16.

Lee IE, Chuyen NV, Hayase F, Kato H. 1994. Desmutagenicity of melanoidins against various kinds of mutagens and activated mutagens. Biosci Biotechnol Biochem 58:18–23.

Leifer Z, Kada T, Mandel M. 1981. An evaluation of tests using DNA repair-deficient bacteria for predicting genotoxicity and carcinogenicity. A report of the U.S. EPA's Gene-Tox Program. Mutat Res 87:211–297.

Leo A, Hansch C, Elkins D. 1971. Partioning coefficents and their uses. Chem Rev 71:525-616.

Lewis RJ Sr, Sax NI. 2000. Sax's dangerous properties of industrial materials (10th ed.). Volume 1. J Wiley. University of Michigan.

Lide DR, editor. 2005–2006. CRC handbook of chemistry and physics. 86th ed. Boca Raton (FL): CRC Press.

Lin JK, Wu YH. 1973. Studies on the mechanism of methemoglobin formation induced by aminoazo compounds. Biochem Pharmacol 22:1883–1891.

Lin JK, Wu JR. 1974. Syntheses, toxicities, and carcinogenicities of carcinogenic bifunctional and aminoazo dyes. Cancer Res 34:2274–2282.

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

Litton Bionetics Inc. 1982. Evaluation of C-331 in the *in vitro* transformation of BALB/3T3 cells assay / Evaluation of C-331 in the mouse lymphoma forward mutation assay / Evaluation of C-331 in the Ames *Salmonella*/microsome plate test. Frederick (MD): Litton Bionetics Inc.

[LNHPD] Licensed Natural Health Products Database [database on the Internet]. 2008. Version 1.0. Ottawa (ON): Health Canada. [cited 2012 Dec]. Available from: webprod3.hc-sc.gc.ca/lnhpd-bdpsnh/index-eng.jsp

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

LookChem [database on the Internet]. ©2008. Look for Chemicals. [cited 2013 Jan]. Available from: http://www.lookchem.com/

Lopes TJ, Furlong ET. 2001. Occurrence and potential adverse effects of semivolatile organic compounds in stream bed sediments, United States, 1992-95. Environ Toxicol Chem 20(4):727–737.

Loretz LG, Api AM, Barraj LM, Burdick J, Dressler WE, Gettings SD, Han Hsu H, Pan YHL, Re TA, Renskers KJ, Rothenstein A, Scrafford CG, Sewall C. 2005. Exposure data for cosmetic products: lipstick, body lotion, and face cream. Food Chem Toxicol 43:279–291.

Loretz L, Api AM, Barraj L, Burdick J, Davis DA, Dressler W, Gilberti E, Jarrett G, Mann S, Pan YHL, Re T, Renskers K, Scrafford C, Vater S. 2006. Exposure data for personal care products: hairspray, spray perfume, liquid foundation, shampoo, body wash, and solid antiperspirant. Food Chem Toxicol 44: 2008–2018.

Loretz LG, Api AM, Babcock L, Barraj LM, Burdick J, Cater KC, Jarrett G, Mann S, Pan YHL, Re TA, Renskers KJ, Scrafford CG. 2008. Exposure data for cosmetic products: facial cleanser, hair conditioner, and eye shadow. Food Chem Toxicol 46:1516–1524.

Lubet RA, Connolly G, Kouri RE, Nebert DW, Bigelow SW. 1983. Biological effects of the Sudan dyes. Role of the AH cytosolic receptor. Biochem Pharmacol 32:3053–3058.

Lundsgaard J. 2002. Investigations of pigments in tattoo colours. (Survey of chemical compounds in consumer products, Survey no. 2). Copenhagen (DK): Ministry of Environment and Energy, Danish Environmental Protection Agency. Available from: www.mst.dk/NR/rdonlyres/FCB88CDC-3C3C-4BF5-AB5F-51E32BA994A8/0/2.pdf

Macholz R, Kujawa M, Schulze J, Lewerenz HJ, Schnaak W. 1985. The metabolism of some xenobiotics in germ-free and conventional rats. Arch Toxicol Suppl 8:373–376.

Maguire RJ, Tkacz RJ. 1991. Occurrence of dyes in the Yamaska River, Quebec. Water Pollut Res J Can 26(2):145–161.

Majlathova L, Rippel A. 1970. Effects of chronic feeding of Sudan Yellow 3G to mice and rats. Cesk Hyg 15:143–146.

Malaney GW. 1960. Oxidative abilities of aniline-acclimated activated sludge. J Water Poll Control Fed 32(12):1300–1311

Mamber SW, Bryson V, Katz SE. 1984. Evaluation of the *Escherichia coli* K12 inductest for detection of potential chemical carcinogens. Mutat Res 130:141–151.

Mamber SW, Okasinski WG, Pinter CD, Tunac JB. 1986. The *Escherichia coli* K-12 SOS chromotest agar spot test for simple, rapid detection of genotoxic agents. Mutat Res 171:83–90.

Manam S, Storer RD, Prahalada S, Leander KR, Kraynak AR, Ledwith BJ, van Zwieten MJ, Bradley MO, Nichols WW. 1992a. Activation of the Ha-, Ki-, and N-*ras* genes in chemically induced liver tumors from CD-1 mice. Cancer Res 52:3347–3352.

Manam S, Storer RD, Prahalada S, Leander KR, Kraynak AR, Hammermeister CL, Joslyn DJ, Ledwith BJ, van Zwieten MJ, Bradley MO, Nichols WW. 1992b. Activation of the Ki-*ras* gene in spontaneous and chemically induced lung tumors in CD-1 mice. Mol Carcinog 6:68–75.

Manthei JH, Lee FK, Heitkamp DH, Heyl WC. 1983. Preliminary and acute toxicological evaluation of five candidate smoke compounds. Edgewood (MD): US Army Armament Research and Development Command, Aberdeen Proving Ground. Technical Report ARCSL-TR-82066. [cited in US NRC 1999].

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

Maruya H, Tanaka Y. 1936. Buttergelb. Osaka Igakkai Zasshi 35:2304. [cited in IARC 1975f].

Matsumoto K, Usui A, Ochiai T, Sekita K, Kawasaki Y, Naito K, Nakaji Y, Furuya T, Tobe M. 1986. Short-term toxicity study of 4-dimethylaminoazobenzene in marmosets. J Toxicol Sci 11:293–301.

Matsumoto M, Terayama H. 1961. Studies on the mechanism of liver carcinogenesis by certain aminoazo dyes. V. *N*-Demethylation of various aminoazobenzene derivatives by rat liver homogenate, with respect to the carcinogenic potency. Gann 52:239–245.

Matsuoka A, Hayashi M, Ishidate MJR. 1979. Chromosomal aberration tests on 29 chemicals combined with S9 mix *in vitro*. Mutat Res 66:277–290.

Matsushima T, Teichmann B, Sawamura M, Sugimura T. 1978. Mutagenicity of azo-compounds. Improved method for detecting their mutagenicities by the *Salmonella* mutation test. Mutat Res 54:220–221.

Matthews EJ, Spalding JW, Tennant RW. 1993. Transformation of BALB/c-3T3 cells: V. Transformation responses of 168 chemicals compared with mutagenicity in *Salmonella* and carcinogenicity in rodent bioassays. Environ Health Perspect 101(Suppl 2):347–482.

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

McCarty LS. 1986. The relationship between aquatic toxicity QSARs and bioconcentration for some organic chemicals. Environ Toxicol Chem. 5:1071–1080

McCarty LS. 1987a. Relationship between toxicity and bioconcentration for some organic chemicals: I Examination of the relationship. In: QSAR in Environmental Toxicology-II, KLE Kaiser (ed). D Reidel Publishing Co, Dordecht, The Netherlands . Pp. 207–220.

McCarty LS. 1987b. Relationship between toxicity and bioconcentration for some organic chemicals: II Application of the relationship. In: QSAR in Environmental Toxicology-II, KLE Kaiser (ed). D Reidel Publishing Co, Dordecht, The Netherlands . Pp. 221–229.

McCarty LS. 1990. A kinetics-based analysis of quantitative structure-activity relationships in aquatic toxicity and bioconcentration bioassays with organic chemicals. Ph.D. thesis. University of Waterloo. Waterloo, Ontario, Canada.

McCarty LS, Mackay D. 1993. Enhancing ecotoxicological modeling and assessment: critical body residues and modes of toxic action. Environ Sci Technol 27:1719–1728.

McCarty LS, Hodson PV, Craig GR, Kaiser KLE. 1985. On the use of quantitative structure-activity relationships to predict the acute and chronic toxicity of chemicals to fish. Environ Toxicol Chem. 4:595–606.

McCarty LS, Mackay D, Smith AD, Ozburn GW, Dixon DG. 1991. Interpreting aquatic toxicity QSARs: the significance of toxicant body residues at the pharmacologic endpoint. Science of the Total Environment, Special Issue: QSAR in Environmental Toxicology 109:515–525.

McDougall R, Van Ham MD. 2002. Best management practice guidelines for the land application of managed organic matter in British Columbia. Victoria (BC): Ministry of Water, Land, and Air Protection.

McGregor DB, Brown A, Cattanach P, Edwards I, McBride D, Riach C, Caspary WJ. 1988. Responses of the L5178Y *tk+/tk−* mouse lymphoma cell forward mutation assay: III. 72 coded chemicals. Environ Mol Mutagen 12:85–154.

McGregor DB, Brown AG, Howgate S, McBride D, Riach C, Caspary WJ. 1991. Responses of the L5178Y mouse lymphoma cell forward mutation assay. V: 27 coded chemicals. Environ Mol Mutagen 17:196–219.

Meara Rh, Martin-Scott I. 1953. Contact dermatitis due to aminoazotoluene. Br Med J 1(4820):1142–1143.

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

[MENV] Ministère de l'Environnement du Québec. 2004. Guidelines for the beneficial use of fertilizing residuals. February.

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

Mersch-Sundermann V, Schneider U, Klopman G, Rosenkranz HS. 1994. SOS induction in *Escherichia coli* and *Salmonella* mutagenicity: a comparison using 330 compounds. Mutagenesis 9:205–224.

Meylan WM, Howard PH. 1995. Atom/fragment contribution method for estimating octanol-water partition coefficients. J Pharm Sci 84:83–92.

Meylan WM, Howard PH, Boethling RS. 1996. Improved method for estimating water solubility from octanol/water partition coefficient. Environ Toxicol Chem 15:100–106.

Mikhailova ON, Vasyunina EA, Ovchinnikova LP, Gulyaeva LF, Timofeeva OA, Filipenko ML, Kaledin VI. 2005. *o*-Aminoazotoluene does induce the enzymes of its own mutagenic activation in mouse liver. Toxicology 211:132–138.

Milani D. 2013. Preliminary results of a range-finding study on the toxicity of the monoazo pigment Sudan Red G in soil to turtle embryos (*Chelydra serpentina*). Internal report (unpublished) prepared by the Watershed Hydrology and Ecology Research Division, Water Science and Technology Directorate, Environment Canada. Submitted to the Emerging Priorities Division, Science and Risk Assessment Directorate, Environment Canada. March 25, 2013. 29p.

Milani D, Bartlett A, Intini K, Balakrishnan V, Webber J. 2014. Toxicity of the monoazo pigment Sudan Red G to the mayfly *Hexagenia* spp. and oligochaete worm *Tubifex tubifex* in spiked sediment exposures. Internal report (unpublished) prepared by the Water Science and Technology Directorate, Environment Canada. Submitted to the Emerging Priorities Division, Science and Risk Assessment Directorate, Environment Canada. Updated June 2014. 41p.

Miller EC, Kadlubar FF, Miller JA, Pitot HC, Drinkwater NR. 1979. The *N*-hydroxy metabolites of *N*-methyl-4-aminoazobenzene and related dyes as proximate carcinogens in the rat and mouse. Cancer Res 39:3411–3418.

Miller JA, Miller EC. 1948. The carcinogenicity of certain derivatives of *p*-dimethylaminozobenz in the rat. J Exp Med 87:139–156.

Miller JA, Miller EC, Baumann C. 1945. On the methylation and demethylation of certain carcinogenic azo dyes in the rat. Cancer Res 5:162–168.

Mirsalis J, Tyson K, Loh E, Bakke J, Hamilton C, Spak D, Steinmetz K, Spalding J. 1985. Induction of unscheduled DNA synthesis and cell proliferation in mouse and rat hepatocytes following in-vivo treatment. [16th Annual Meeting of the Environmental Mutagen Society, Las Vegas, NV, February 25 – March 1, 1985]. Environ Mutagen 7(Suppl 3):73 (abstract).

Mirsalis JC, Tyson CK, Steinmetz KL, Loh EK, Hamilton CM, Bakke JP, Spalding JW. 1989. Measurement of unscheduled DNA synthesis and S-phase synthesis in rodent hepatocytes following *in vivo* treatment: testing of 24 compounds. Environ Mol Mutagen 14:155–164.

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

[MITI] Ministry of International Trade & Industry (JP), Basic Industries Bureau, Chemical Products Safety Division. 1992. Biodegradation and bioaccumulation data of existing chemicals based on the CSCL Japan. Tokyo (JP): Japan Chemical Industry Ecology-Toxicology & Information Centre. As cited in OECD QSAR Toolbox (2012).

Miyagoshi M, Hayakawa Y, Nagayama T. 1983. [Studies on the mutagenicity of cosmetic azo-dyes]. Eisei Kagaku 29:212–220. [in Japanese].

Miyagoshi M, Hayakawa Y, Nagata M, Nagayama T. 1985. Mutagenic activities of commercial Sudan III (Solvent Red 23) and Scarlet Red are due to impurities. Eisei Kagaku 31(2):79–86.

[MOE, OMAFRA] Ontario Ministry of Environment and Ontario Ministry of Agriculture, Food, and Rural Affairs. 1996. Guidelines for the utilization of biosolids and other wastes on agricultural land. Available from:
www.ene.gov.on.ca/stdprodconsume/groups/lr/@ene/@resources/documents/resource/std01_079003.pdf

Moore MM, Claxton L, Houk V, Nelson GM, Harrington-Brock K. 1989. Toxicity of red and violet dyes in M18 grenades: mutagenic screening of three dyes for marker grenades in the *Salmonella* reversion assay and the L5178Y/TK$^{+/-}$ mouse lymphoma assay. Final report. AD-A212965. Research Triangle Park (NC): US Environmental Protection Agency, Health Effects Research Laboratory.

Mori H, Mori Y, Sugie S, Yoshimi N, Takahashi M, Ni-i H, Yamazaki H, Toyoshi K, Williams GM. 1986. Genotoxicity of a variety of azobenzene and aminoazobenzene compounds in the hepatocyte/DNA repair test and the *Salmonella*/mutagenicity test. Cancer Res 46:1654–1658.

Mori K, Nakahara W. 1940. Effect of feeding liver on the production of malignant tumors by injections of carcinogenic substances. Gann 34:48.

Mori K, Ichii S, Sigeta Y. 1956. Inhibition of experimental production of liver cancer by tobacco tar. Gann 47:97–103.

Morita T, Asano N, Awogi T, Sasaki YF, Sato S, Shimada H, Sutou S, Suzuki T, Wakata A, Sofuni T, Hayashi M. 1997a. Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B). The summary report of the 6th collaborative study by CSGMT/JEMS MMS. Mutat Res 389:3–122.

Morita T, Asano N, Awogi T, Sasaki YF, Sato S, Shimada H, Sutou S, Suzuki T, Wakata A, Sofuni T, Hayashi M. 1997b. Erratum to “Evaluation of the rodent micronucleus assay in the screening of IARC carcinogens (groups 1, 2A and 2B). The summary report of the 6th collaborative study by CSGMT/JEMS.MMS.” Mutat Res 391:259–267.

Morosenskaya LS. 1938. *Ortho*-aminoazotoluene. Arch Sci Biol Moscow 56:189–200. [cited in IARC 1975b].

Morosenskaya LS. 1939. On the local effect of ortho aminoazotoluene and the distant tumours elicited by it outside the liver. Arch Sci Biol Moscow 56:53–58. [cited in IARC 1975b].

MP Biomedicals, LLC. 2006. Material Safety Data Sheet: Fat Brown B (6535-42-8) [Internet]. Solon (OH): MSDSonline. [cited April 2011]. [restricted access]

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

Mueller GC, Miller JA. 1949. The reductive cleavage of 4-dimethylaminoazobenzene by rat liver; the intracellular distribution of the enzyme system and its requirement for triphosphopyridine nucleotide. J Biol Chem 180:1125–1136.

Muller D, Nelles J, Deparade E, Arni P. 1980. The activity of S9-liver fractions from seven species in the *Salmonella*/mammalian-microsome mutagenicity test. Mutat Res 70:279–300.

Müller VM. 1967. Autoradiographische, fluoreszenzimmunologische und morphologische untersuchungen zur verteilung und Wirkung von o-amino-azotoluol (OAAT) in der mäuseleber. Arch Geschwulstforsch 30:97–108. [in German; cited in IARC 1975b].

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

Nagao M, Yahagi T, Honda M. 1977. Comutagenic actions of norharman derivatives with 4-dimethylaminoazobenzene and related compounds. Cancer Lett 3:339–346.

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

Nakayama T, Kimura T, Kodama M, Nagata C. 1983. Generation of hydrogen peroxide and superoxide anion from active metabolites of naphthylamines and aminoazo dyes: its possible role in carcinogenesis. Carcinogenesis 4:765–769.

Nardelli A, Degreef H, Goossens A. 2004. Contact allergic reactions of the vulva: a 14-year review. Dermatitis 15:131–136.

Nardelli A, Taveirne M, Drieghe J, Carbonez A, Degreef H, Goossens A. 2005. The relation between the localization of foot dermatitis and the causative allergens in shoes: a 13-year retrospective study. Contact Dermatitis 53:201–206.

[NCI] National Cancer Institute (US). 1978. Bioassay of *o*-anisidine hydrochloride for possible carcinogenicity. Bethesda (MD): NCI. NCI Carcinogenesis Technical Report Series No. 89. Available from: http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr089.pdf

[NCI] National Cancer Institute (US). 1979. Bioassay of azobenzene for possible carcinogenicity. Bethesda (MD): NCI. NCI Carcinogenesis Technical Report Series No. 154. Available from: http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr154.pdf

[NCI] National Chemical Inventories [database on a CD-ROM]. 2012. Issue 2. Columbus (OH): American Chemical Society, Chemical Abstracts Service. [cited 2012 and 2013]. Available from: www.cas.org/products/other-cas-products/nci-on-cd

Nelson AA, Davidow B. 1957. Injection site fibrosarcoma production in rats by food colors. Fed Proc 16:367.

Nelson AA, Woodard G. 1953. Tumors of the urinary bladder, gall bladder, and liver in dogs fed *o*-aminoazotoluene or *p*-dimethylaminoazobenzene. J Natl Cancer Inst 13:1497–1509.

Neumann HG. 2005. Monocyclic aromatic amino and nitro compounds: toxicity, genotoxicity and carcinogenicity, classification in a carcinogen category. In: The MAK-collection Part I: MAK value documentations, vol. 21. Weinheim (DE): Deutsche Forschungsgemeinschaft (DFG); Wiley-VCH Verlag GmbH & Co. KGaA.

[NHPID] Natural Health Products Ingredients Database [database on the Internet]. 2011. Version 2.1. Ottawa (ON): Health Canada. [cited 2012 Oct]. Available from: http://webprod.hc-sc.gc.ca/nhpid-bdipsn/search-rechercheReq.do

[NICNAS] National Industrial Chemicals Notification and Assessment Scheme (AU). 2009. Triclosan [Internet]. Sydney (AU): Department of Health and Ageing. Priority Existing Chemical Assessment Report No. 30. Available from: www.nicnas.gov.au/publications/car/pec/pec30/pec_30_full_report_pdf.pdf

Niemi GJ, Veith GD, Regal RR, Vaishnav DD. 1987. Structural features associated with degradable and persistent chemicals. Environ Toxicol Chem 6:515–527

Nishida K, Ando Y, Ohwada K, Mori T, Koide M, Koukitsu . 1989. Vapour pressures and heats of sublimation of some azo disperse dyes. J Soc Dyers Colorists 105:112–114.

Nishizuka Y, Ito K, Nakakuki K. 1965. Liver tumor induction by a single injection of *o*-aminoazotoluene to newborn mice. Gann 56:135–142.

Noon AP, Pickvance SMJ, Catto JWF. 2012. Occupational exposure to crack detection dye penetrants and the potential for bladder cancer. Occup Environ Med 69:300–301.

[NTP] National Toxicology Program (US). 1978. Bioassay of *p*-anisidine hydrochloride for possible carcinogenicity (CAS No. 20265-97-8). Research Triangle Park (NC): US Department of Health and Human Services, National Toxicology Program. Technical Report Series No. 116.

[NTP] National Toxicology Program (US). 1982a. Carcinogenesis bioassay of Disperse Yellow 3 (CAS No. 2832-40-8) in F344 rats and B6C3F1 mice (feed) study. Research Triangle Park (NC): US Department of Health and Human Services, National Toxicology Program. Technical Report Series No. 222.

[NTP] National Toxicology Program (US). 1982b. Carcinogenesis bioassay of C.I. Solvent Yellow 14 (CAS No. 842-07-9) in F344/N rats and B6C3F1 mice (feed study). Research Triangle Park (NC): US Department of Health and Human Services, National Toxicology Program. Technical Report Series No. 226.

[NTP] National Toxicology Program (US). 1983. Study results for *Salmonella* Ames test for CAS RN 2832-40-8 [Internet]. Available from: http://tools.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=salmonella.salmonellaData&endpointlist=SA&study_no=376537&cas_no=2832-40-8&activetab=detail

[NTP] National Toxicology Program (US). 1985. Study results for sister chromatid exchanges test for CAS RN 2832-40-8 [Internet]. Available from:

http://tools.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=invitrosce.scedata&study_no=041446&cas_no=2832-40-8&endpointlist=SCE

[NTP] National Toxicology Program (US). 1988a. *Salmonella* results for azobenzene, CAS RN 103-33-3, Study ID: 513062 [Internet]. Available from: http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=salmonella.salmonellaData&study_no=513062&cas_no=103-33-3&endpointlist=SA.

[NTP] National Toxicology Program (US). 1988b. *Salmonella* results for C.I. Solvent Yellow 14, CAS RN 842-07-9, Study ID: 916978 [Internet]. Available from: http://tools.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=salmonella.salmonellaData&endpointlist=SA&study_no=916978&cas_no=842-07-9&activetab=detail

[NTP] National Toxicology Program (US). 1993b. Study results for *Salmonella* Ames test for CAS RN 2832-40-8 [Internet]. Available from: http://tools.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=salmonella.salmonellaData&endpointlist=SA&study_no=328289&cas_no=2832-40-8&activetab=detail

[NTP] National Toxicology Program (US). 2011. Report on carcinogens. 12th ed. Research Triangle Park (NC): US Department of Health and Human Services, National Toxicology Program. Available from: http://ntp.niehs.nih.gov/ntp/roc/twelfth/roc12.pdf

[NTP] National Toxicology Program (US). [undated-a]. Chromosome aberrations results for azobenzene, CAS RN 103-33-3, Study ID: 758475 [Internet]. Available from: http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=invitroca.cadata&study_no=758475&cas_no=103-33-3&endpointlist=CAB

[NTP] National Toxicology Program (US). [undated-b]. Study results for mouse lymphoma test for CAS RN 2832-40-8, Study ID: 571100 [Internet]. Available from: http://tools.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=mouselymphoma.studyDetails&study_no=571100&cas_no=2832-40-8&endpointlist=ML,ML-N

[NTP] National Toxicology Program (US). [undated-c]. Micronucleus study results for C.I. Solvent Yellow 14, CAS RN 842-07-9, Study ID: 666998 [Internet]. Available from: http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=micronucleus.micronucleusData&current_strain_id=B6C3F1&endpointlist=MN&cas_no=842-07-9&study_no=666998&activetab=detail

[NTP] National Toxicology Program (US). [undated-d]. Sister chromatid exchange study results for C.I. Solvent Yellow 14, CAS RN 842-07-9, Study ID: 114547 [Internet]. Available from: http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=invivosc.scsummary&study_no=114547&cas_no=842-07-9&endpointlist=SC.

[NTP] National Toxicology Program (US). [undated-e]. Chromosome aberrations study results for C.I. Solvent Yellow 14, CAS RN 842-07-9, Study ID: 114547 [Internet]. Available from: http://ntp-apps.niehs.nih.gov/ntp_tox/index.cfm?fuseaction=invivo.choosestudytype&cas_no=842-07-9&endpointlist=CA%2CSC

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

Odashima S, Hashimoto Y. 1968. Carcinogenicity and target organs of methoxyl derivatives of 4-aminoazobenzene in rats. I. Gann 59:131–43.

[OECD] Organisation for Economic Co-operation and Development. 2002. SIDS Final Assessment Report for: *p*-Phenetidine; CAS RN 156-43-4. Available from: http://webnet.oecd.org/Hpv/UI/handler.axd?id=bc1f4c84-04bb-4bf6-b74b-a45419deebbf

[OECD] Organisation for Economic Co-operation and Development. 2012. SIDS Initial Assessment Profile (SIAP) for Dimethylaniline Category. CoCAM [Cooperative Chemicals Assessment Meeting] 3, 16–18 October 2012. Available from: http://webnet.oecd.org/HPV/UI/handler.axd?id=facd5716-0e31-43e2-ba9f-6171b5097489

[OECD] Organisation for Economic Co-operation and Development. 2014. Guidance on Grouping of Chemicals. Second Edition. Series on Testing & Assessment No. 194. Available at: http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2014)4&doclanguage=en

OECD QSAR Toolbox [Read-across tool]. 2012. Version 3.0. Paris (FR): Organisation for Economic Co-oporation and Development, Environment Directorate. Available from: www.oecd.org/document/23/0,3343,en_2649_34379_33957015_1_1_1_1,00.html

Ohsawa K, Hirano N, Sugiura M, Nakagawa S, Kimura M. 2000. Genotoxicity of *o*-aminoazotoluene (AAT) determined by the Ames test, the *in vitro* chromosomal aberration test, and the transgenic mouse gene mutation assay. Mutat Res 471:113–126.

Øllgaard H, Frost L, Galster J, Hansen OC. 1998. Survey of azo-colorants in Denmark: consumption, use, health and environmental aspects. Copenhagen (DK): Ministry of Environment and Energy, Danish Environmental Protection Agency. Available from: www2.mst.dk/udgiv/publications/1999/87-7909-548-8/pdf/87-7909-546-1.pdf

Orr J. 1940. The histology of the rat's liver during the course of carcinogenesis by butter-yellow (*p*-dimethylaminoazobenzene). J Pathol Bacteriol 50:393.

Özacar M, Şengil İA. 2002. Adsorption of acid dyes from aqueous solutions by calcined alunite and granular activated carbon. Adsorption 8(4):301–308.

Pakharukova M. 2011. OAT and 3′MeDAB azo compounds similarly cause liver tumors in GR mice, but differently modify activities of FoxA transcription factors. Bull Exp Biol Med 152:101–104. [in English, Russian].

Pan H, Feng J, He G, Cerniglia C, Chen H. 2012. Evaluation of impact of exposure of Sudan azo dyes and their metabolites on human intestinal bacteria. Anaerobe 18:445–453.

Parent RA, Dressler I. 1979. Absorption and distribution of C.I. Solvent Red 24 in rats; intra-tracheal administration of ^{14}C labeled dye. Drug Chem Toxicol 2:409–420. [cited in EFSA 2005].

Parodi S, Taningher M, Russo P, Pala M, Tamaro M, Monti-Bragadin C. 1981. DNA-damaging activity *in vivo* and bacterial mutagenicity of sixteen aromatic amines and azo-derivatives, as related quantitatively to their carcinogenicity. Carcinogenesis 2:1317–1326.

Parodi S, Zunino A, Ottaggio L, De Ferrari M, Santi L. 1983. Lack of correlation between the capability of inducing sister-chromatid exchanges *in vivo* and carcinogenic potency, for 16 aromatic amines and azo derivatives. Mutat Res 108:225–238.

Parrott J, Bartlett A, Hill J, Balakrishnan V. 2014. Chronic toxicity of azo and anthracenedione dyes to embryo-larval fathead minnow. Internal report (unpublished) prepared by the Aquatic Contaminants Research Division, Water Science and Technology Directorate, Environment Canada. Submitted to the Emerging Priorities Division, Science and Risk Assessment Directorate, Environment Canada. January, 2014. 31p.

Patterson D, Sheldon RP. 1960. The solubilities and heats of solution of disperse dyes in water. J Soc Dyers Colourists 76(3):178–181.

Pereira L, Coelho AV, Viegas CA, Santos MM, Robalo MP, Martins LO. 2009. Enzymatic biotransformation of the azo dye Sudan Orange G with bacterial CotA-laccase. J Biotechnol 139:68–77.

Perrin DD. 1972. Dissociation constants of organic bases in aqueous solution. Buttersworth (London): IUPAC Chemical Data Series: Supplement 1972. As cited in EPI Suite 2012.

Perry PE, Thomson EJ. 1981. Evaluation of the sister chromatid exchange method in mammalian cells as a screening system for carcinogens. Prog Mutat Res 1:560–569.

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

Pielesz A, Baranowska I, Rybak A, Wlochowicz A. 2002. Detection and determination of aromatic amines as products of reductive splitting from selected azo dyes. Ecotox Environ Safety 53:42–47.

Pienta RJ. 1980a. Evaluation and relevance of the Syrian hamster embryo cell system. In: Williams GM, Kroes R, Waaijers HW, van de Poll KW, editors. The predictive value of short-term screening tests in carcinogenicity evaluation. Amsterdam (NL): Elsevier; p. 149–169.

Pienta RJ. 1980b. Transformation of Syrian hamster embryo cells by diverse chemicals and correlation with their reported carcinogenic and mutagenic activities. In: Hollaender A, de Serres FJ, editors. Chemical mutagens: principles and methods for their detection, vol. 6. New York (NY): Plenum; p. 175–202.

Pitot HC, Goodspeed D, Dunn T, Hendrich S, Maronpot RR, Moran S. 1989. Regulation of the expression of some genes for enzymes of glutathione metabolism in hepatotoxicity and hepatocarcinogenesis. Toxicol Appl Pharmacol 97:23–34.

Poiley JA, Raineri R, Pienta RJ. 1979. Use of hamster hepatocytes to metabolize carcinogens in an *in vitro* bioassay. J Natl Cancer Inst 63:519–524.

Poirier LA, Miller JA, Miller EC, Sato K. 1967. *N*-Benzoyloxy-*N*-methyl-4-aminoazobenzene: its carcinogenic activity in the rat and its reactions with proteins and nucleic acids and their constituents *in vitro*. Cancer Res 27:1600–1613.

Popova NV. 1989. Transgenerational effect of orthoaminoasotoluol in mice. Cancer Lett 46:203–206.

Pritchard DJ, Butler WH. 1984. Comparative study of development of *ortho*-aminoazotoluene induced hepatocellular carcinoma in C3H and C-57B1 mice. [149th Meeting of the Pathological Society of Great Britain and Ireland, July 4–6, 1984, Leeds, England]. J Pathol 143:319 (abstract).

Probst GS, Hill LE. 1980. Chemically-induced DNA repair synthesis in primary rat hepatocytes: a correlation with bacterial mutagenicity. Ann NY Acad Sci 349:405–406.

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

Radding SB, Liu DH, Johnson HL, Mill JT. 1977 Review of the environmental fate of selected chemicals. Washington (D.C.): Prepared for U.S. EPA, Office of Toxic Substances. Final Report (EPA-560/5-77-003, May 1977). 148 pp.

Radomski JL, Deichmann WB. 1956. Cathartic action and metabolism of certain coal tar food dyes. J Pharmacol Exp Ther 118:322–327.

Raevsky OA, Dearden JC. 2004. Creation of predictive models of aquatic toxicity of environmental pollutants with different mechanisms of action on the basis of molecular similarity and HYBOT descriptors. SAR QSAR Environ Res 15(5–6):433–448.

Rahimtula A, Moldeus P, Andersson B, Nordenskjold M. 1982. Prostaglandin synthetase catalyzed DNA strand breaks by aromatic amines. In: Powles T, editor. Prostaglandins and related lipids, vol. 2. Prostaglandins and cancer. [First International Conference, August 30 – September 2, 1981, Washington, DC]. New York (NY): Alan R. Liss, Inc.; p. 159.

RAPEX [European Union rapid alert system]. 2012. RAPEX—Latest notifications [Internet]. Brussels (BE): European Commission, Directorate-General Health & Consumers. [cited 2012 Jul 12]. Available from: http://ec.europa.eu/consumers/dyna/rapex/rapex_archives_en.cfm

[RASFF] Rapid Alert System for Food and Feed [Internet]. 2012. Brussels (BE): European Commission, Directorate-General Health & Consumers. [cited 2012 Dec 12]. Available from: http://ec.europa.eu/food/food/rapidalert/index_en.htm

ReagentWorld Inc. 2013. Material Safety Data Sheet: Direct Red 81 (2610-11-9; dye content 50%) [Internet]. Ontario (CA) [cited April 2013]. Available from: https://www.reagentworld.com/products/msds2.asp?proid_2=31072

Refat NAGA, Ibrahim ZS, Moustafa GG, Sakamoto KQ, Ishizuka M, Fujita S. 2008. The induction of cytochrome P450 1A1 by Sudan dyes. J Biochem Mol Toxicol 22:77–84.

Reifferscheid G, Heil J. 1996. Validation of the SOS/umu test using test results of 486 chemicals and comparison with the Ames test and carcinogenicity data. Mutat Res 369:129–145.

Rinkus S, Legator M. 1979. Chemical characterization of 465 known or suspected carcinogens and their correlation with mutagenic activity in the *Salmonella typhimurium* system. Cancer Res 39:3289–3318.

[RIVM] Rijksinstituut voor Volksgezondheid en Milieu [National Institute for Public Health and the Environment (NL)]. 2006a. Cosmetics fact sheet: to assess the risks for the consumer. Updated version for ConsExpo 4 [Internet]. Bilthoven (NL): RIVM (National Institute for Public Health and the Environment). Report No.: 320104001/2006. Available from: www.rivm.nl/bibliotheek/rapporten/320104001.pdf

[RIVM] Rijksinstituut voor Volksgezondheid en Milieu [National Institute for Public Health and the Environment (NL)]. 2006b. Cleaning products fact sheet: to assess the risks for the consumer [Internet]. Bilthoven (NL): RIVM (National Institute for Public Health and the Environment). Report No: 320104003/2006. Available from: www.rivm.nl/bibliotheek/rapporten/320104003.pdf

[RIVM] Rijksinstituut voor Volksgezondheid en Milieu [National Institute for Public Health and the Environment (NL)]. 2010. New default values for the spray model [Internet]. Bilthoven (NL): RIVM (National Institute for Public Health and the Environment). [cited 2012 Jul 27]. Available from: www.rivm.nl/dsresource?type=pdf&objectid=rivmp:60686&versionid=&subobjectname=

Roberts J, Warwick G. 1966a. The covalent binding of metabolites of dimethylaminoazobenzene, β-naphthylamine and aniline to nucleic acids *in vivo*. Int J Cancer 1:179–196.

Roberts J, Warwick G. 1966b. Covalent binding of 4-dimethylaminophenylazo-^{3}H-benzene (butter yellow) metabolites with liver ribosomal RNA: the dissociation of the binding mechanism from the orotic acid incorporating system. Int J Cancer 1:573–578.

Robinson PJ, Ryan AJ, Wright SE. 1964. Metabolism of some dimethylaminoazobenzene derivatives. J Pharm Pharmacol 16(Suppl 1):80T–82T.

Rodrigues AD, Ayrton AD, Williams EJ, Lewis DF, Walker R, Ioannides C. 1989. Preferential induction of the rat hepatic P450 I proteins by the food carcinogen 2-amino-3-methyl-imidazo[4,5-f]quinoline. Eur J Biochem 181:627–631.

Roe FJC, Warwick GP, Carter RL, Peto R, Ross WCJ, Mitchtoy BCV, Barren NA. 1971. Liver and lung tumors in mice exposed at birth to 4-dimethylaminoazobenzene or its 2-methyl or 3′-methyl derivatives. J Natl Cancer Inst 47:593–601.

Rosenkranz HS, Leifer Z. 1980. Determining the DNA-modifying activity of chemicals using DNA-polymerase-deficient *Escherichia coli.* Chem Mutagen 6:109–147.

Rosenkranz HS, Poirier LA. 1979. Evaluation of the mutagenicity and DNA-modifying activity of carcinogens and noncarcinogens in microbial systems. J Natl Cancer Inst 62:873–892.

Roussy G, Guérin M. 1946. Recherches sur l'action cancérogène de certaines substances colorantes. Bull Acad Med (Paris) 130:156–161.

Rufli H, Fisk PR, Girling AE, King MH, Lange R, Lejeune X, Stelter N, Stevens C, Suteau P, Tapp J, Thus HJ, Versteeg DJ, Niessen HJ. 1998. Aquatic toxicity testing of sparingly soluble, volatile, and unstable substances and interpretation and use of data. Ecotoxicol Environ Saf 39:72–77.

Russom CL, Bradbury SP, Broderius SJ, Hammermeister DE, Drummond RA. 1997. Predicting modes of toxic action from chemical structure: acute toxicity in the fathead minnow (*Pimephales promelas*). Environ Toxicol Chem 16(5):948–967.

Sailstad DM, Tepper JS, Doerfler DL, Selgrade MK. 1993. Evaluation of several variations of the mouse ear swelling test (MEST) for detection of weak and moderate contact sensitizers. Toxicol Methods 3:169–182.

Sailstad DM, Tepper JS, Doerfler DL, Qasim M, Selgrade MK. 1994. Evaluation of an azo and two anthraquinone dyes for allergic potential. Fundam Appl Toxicol 23:569–577.

Sakuratani Y, Noguchi Y, Kobayashi K, Yamada J, Nishihara T. 2008. Molecular size as a limiting characteristic for bioconcentration in fish. J Environ Biol 29(1):89–92.

Salamone MF, Heddle JA, Katz M. 1981. Mutagenic activity of 41 compounds in the *in vivo* micronucleus assay. In: de Serres FJ, Ashby J, editors. Evaluation of short-term tests for carcinogens. (Progress in Mutation Research, vol. I). New York (NY): Elsevier/North-Holland; p. 686–697.

Samejima K, Tamura Z, Ishidate M. 1967. Metabolism of 4-dimethylaminoazobenzene and related compounds. IV. Metabolites of *o*-aminoazotoluene in rat bile. Chem Pharm Bull 15:964–975.

Samuels AR, Bhargava MM, Levine WG. 1983. Uptake and hepatobiliary fate of two hepatocarcinogens, *N*,*N*-dimethyl-4-aminoazobenzene and 3′-methyl-*N*,*N*-dimethyl-4-aminoazobenzene, in the rat. Cancer Res 43:4816–4821.

Sasaki T, Yoshida T. 1935. Liver carcinoma induced by feeding *o*-amidoazotoluene. Virchows Archiv Pathol Anat 295:175–220.

Sasaki YF, Izumiyama F, Nishidate E, Matsusaka N, Tsuda S. 1997. Detection of rodent liver carcinogen genotoxicity by the alkaline single-cell gel electrophoresis (comet) assay in multiple mouse organs (liver, lung, spleen, kidney, and bone marrow). Mutat Res 391:201–214.

Sasaki YF, Sekihashi K, Izumiyama F, Nishidate E, Saga A, Ishida K, Tsuda S. 2000. The comet assay with multiple mouse organs: comparison of comet assay results and carcinogenicity with 208 chemicals selected from the IARC monographs and U.S. NTP carcinogenicity database. Crit Rev Toxicol 30:629–799.

Sato K, Poirier LA, Miller JA, Miller EC. 1966. Studies on the *N*-hydroxylation and carcinogenicity of 4-aminoazobenzene and related compounds. Cancer Res 26:1678–1687.

Sato Y, Katsumura Y, Ichikawa H, Kobayashi T, Kozuka T, Morikawa F, Ohta S. 1981. A modified technique of guinea pig testing to identify delayed hypersensitivity allergens. Contact Dermatitis 7:225–237.

Sax NI. 1986. 2-Amino-5-azotoluene. In: Sax's dangerous properties of industrial materials, vol. 6. New York (NY): Van Nostrand Reinhold; p. 54–62.

[SCC] Scientific Committee on Cosmetology. 1988. Reports of the Scientific Committee on Cosmetology. Seventh series. Commission of the European Communities.

[SCCP] Scientific Committee on Consumer Products. 2006a. The SCCP's notes of guidance for the testing of cosmetic ingredients and their safety evaluation, 6th revision. European Commission, Health & Consumer Protection Directorate-General. Available from: http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_03j.pdf

[SCCP] Scientific Committee on Consumer Products. 2006b. Opinion on *p*-phenylenediamine. COLIPA No. A7. European Commission, Health & Consumer Protection Directorate-General. Report No.: SCCP/0989/06. Available from: http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_069.pdf

Schäfer U, Metz J, Pevny I, Röckl H. 1978. [Attempts to sensitize guinea-pigs with five different derivatives of *para*-substituted benzene]. Arch Dermatol Res 261:153–161. [in German].

Scherr GH, Fishman M, Weaver RH. 1954. The mutagenicity of some carcinogenic compounds for *Escherichia coli*. Genetics 39:141–149.

Schultz TW. 1997. TETRATOX: *Tetrahymena pyriformis* population growth impairment endpoint—a surrogate for fish lethality. Toxicol Methods 7:289–309.

Scorecard [the Pollution Information Site]. 2011. GoodGuide. [cited 2012 Dec]. Available from: http://scorecard.goodguide.com/

Scott B, Moore L. 2000. Assessment of the risks to human health posed by azo colourants in toys, writing inks and paper products, and analysis of the advantages and drawbacks of restrictions on their marketing and use. Final report. United Kingdom Laboratory of the Government Chemist.

Scribner JD, Miller JA, Miller EC. 1965. 3-Methylmercapto-*N*-methyl-4-aminoazobenzene: an alkaline-degradation product of a labile protein-bound dye in the livers of rats fed *N*,*N*-dimethyl-4-aminoazobenzene. Biochem Biophys Res Commun 20:560–565.

[SDA] The Soap and Detergent Association. 2010a. Appendix II-A-1: Dermal exposure parameters to estimate screening exposures to consumer products—North America. In: Consumer product ingredient safety: exposure and risk screening methods for consumer product ingredients. 2nd ed. Washington (DC): SDA.

[SDA] The Soap and Detergent Association. 2010b. Appendix II-A-2: Dermal exposure parameters to estimate screening exposures to consumer products—Europe. In: Consumer product ingredient safety: exposure and risk screening methods for consumer product ingredients. 2nd ed. Washington (DC): SDA.

Seidenari S, Mantovani L, Manzini BM, Pignatti M. 1997. Cross-sensitizations between azo dyes and *para*-amino compound: a study of 236 azo-dye-sensitive subjects. Contact Dermatitis 36:91–96.

Seidenari S, Giusti F, Massone F, Mantovani L. 2002. Sensitization to disperse dyes in a patch test population over a five-year period. Am J Contact Dermatitis 13:101–107.

Sekihashi K, Sasaki T, Yamamoto A, Kawamura K, Ikka T, Tsuda S, Sasaki YF. 2001. A comparison of intraperitoneal and oral gavage administration in comet assay in mouse eight organs. Mutat Res 493:39–54.

Sekihashi K, Yamamoto A, Matsumura Y, Ueno S, Watanabe-Akanuma M, Kassie F, Knasmuller S, Tsuda S, Sasaki YF. 2002. Comparative investigation of multiple organs of mice and rats in the comet assay. Mutat Res 517:53–75.

Semanská M, Dračínskyě M, Martínek V, Hudeček J, Hodek P, Frei E, Stiborová M. 2008. A one-electron oxidation of carcinogenic nonaminoazo dye Sudan I by horseradish peroxidase. Neuro Endocrinol Lett 29:712–716.

Shackleford WM, Cline DM. 1983. An evaluation of automated spectrum matching for survey identification of wastewater components by gas chromatography-mass spectrometry. Analytica Chimica Acta 146:15–27.

Shelby MD, Witt KL. 1995. Comparison of results from mouse bone marrow chromosome aberration and micronucleus tests. Environ Mol Mutagen 25:302–313.

Shelby MD, Erexson GL, Hook GJ, Tice RR. 1993. Evaluation of a three-exposure mouse bone marrow micronucleus protocol: results with 49 chemicals. Environ Mol Mutagen 21:160–179.

Shelton E. 1955. Hepatomas in mice. I. Factors affecting the rapid induction of a high incidence of hepatomas by *o*-amino-azotoluene. J Natl Cancer Inst 16:107–128.

Shibusawa T, Ohya T, Hamayose T. 1977. Studies pertaining to dyeing propoerties of disperse dyes: Part 8. Thermodynamic studies on dissolution processes of disperse dyes in water. Nippon Kagaku Kaishi 10:1536–1542.

Shimada T, Iwasaki M, Martin MV, Guengerich FP. 1989. Human liver microsomal cytochrome P-450 enzymes involved in the bioactivation of procarcinogens detected by *umu* gene response in *Salmonella typhimurium* TA1535/psk1002. Cancer Res 49:3218–3228.

Shimada T, Hayes CL, Yamazaki H, Amin S, Hecht SS, Guengerich FP, Sutter TR. 1996. Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. Cancer Res 56:2979–2884.

Shimizu T, Ohkubo S, Kimura M, Tabata I, Hori T. 1987. The vapour pressures and heats of sublimation of model disperse dyes. J Soc Dyers Colourists 103(3):132–137.

Sigma-Aldrich. 2010. Material Safety Data Sheet [Internet]. Saint Louis (MO): MSDSonline. [cited April 2011]. [restricted access]

Sijm DTHM, Hermens JLM JLM. 2000. Internal effect concentration: link between bioaccumulation and ecotoxicity for organic chemicals. In: The Handbook of Environmental Chemistry. Vol. 2, Part J. Bioaccumulation (ed. by B. Beek). Springer-Verlag Berlin Heidelberg, 2000. Pp. 167–199.

Silverstone H. 1948. The effect of rice diets on the formation of induced and spontaneous hepatomas in mice. Cancer Res 8:309–317.

Simmon VF. 1979a. *In vitro* mutagenicity assays of chemical carcinogens and related compounds with *Salmonella typhimurium*. J Natl Cancer Inst 62:893–899.

Simmon VF. 1979b. *In vitro* assays for recombinogenic activity of chemical carcinogens and related compounds with *Saccharomyces cerevisiae* D3. J Natl Cancer Inst 62:901–910.

Simmon VF, Rosenkranz HS, Zeiger E, Poirier LA. 1979. Mutagenic activity of chemical carcinogens and related compounds in the intraperitoneal host-mediated assay. J Natl Cancer Inst 62:911–918.

Sina JF, Bean CL, Dysart GR, Taylor VI, Bradley MO. 1983. Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. Mutat Res 113:357–391.

Smetanina MA, Pakharukova MY, Kurinna SM, Dong B, Hernandez JP, Moore DD, Merkulova TI. 2011. *Ortho*-Aminoazotoluene activates mouse constitutive androstane receptor (mCAR) and increases expression of mCAR target genes. Toxicol Appl Pharmacol 255:76–85.

Smith MI, Lillie RD, Stohlman EF. 1943. The toxicity and histopathology of some azo compounds as influenced by dietary protein. Public Health Rep 58:304–317.

Smith SH, Doyle GL, Kreuger JC, Mellon KA, Mayhew DA. 1986. Dermal, eye and oral toxicological evaluations. Phase II. Report with Disperse Red 11, Disperse Blue 3, Solvent Red 1, and Red and Violet mixtures. ADA 172758. Woburn (MA): Bioassay System Corp. [cited in US NRC 1999].

Søsted H, Agner T, Andersen KE, Menné T. 2002. 55 cases of allergic reactions to hair dye: a descriptive, consumer complaint–based study. Contact Dermatitis 47:299–303.

Søsted H, Rastogi SC, Andersen KE, Johansen JD, Menné T. 2004. Hair dye contact allergy: quantitative exposure assessment of selected products and clinical cases. Contact Dermatitis 50:344–348.

Spadaro JT, Gold MH, Renganathan V. 1992. Degradation of azo dyes by the lignin-degrading fungus *Phanerochaete chrysospporium*. Appl Environ Microbiol 58(8):2397–2401.

Spalding JW1, French JE, Tice RR, Furedi-Machacek M, Haseman JK, Tennant RW. 1999. Development of a transgenic mouse model for carcinogenesis bioassays: evaluation of chemically induced skin tumors in Tg.AC mice. Toxicol Sci 49(2):241-54.

Spitz S, Maguigan WH, Dobriner K. 1950. The carcinogenic action of benzidine. Cancer 3:789–804. [cited in IARC 2010].

Stenn KS. 1979. Epidermal hyperplasia induced in guinea pig flank skin by intradermal injection of Sudan Red. Dermatologica (Basel) 159:307–315.

Stevenson ES, Dobriner D, Rhoads CP. 1942. The metabolism of dimethylaminoazobenzene (Butter Yellow) in rats. Cancer Res 2:160–167.

Stiborová M, Asfaw B, Frei E, Schmeiser HH, Wiessler M. 1995. Benzenediazonium ion derived from Sudan I forms an 8-(phenylazo)guanine adduct in DNA. Chem Res Toxicol 8:489–498.

Stiborová M, Schmeiser HH, Frei E. 1999. Prostaglandin H synthase–mediated oxidation and binding to DNA of a detoxication metabolite of carcinogenic Sudan I, 1-(phenylazo)-2,6-dihydroxynaphthalene. Cancer Lett 146:53–60.

Stiborová M, Martínek V, Schmeiser HH, Frei E. 2006. Modulation of CYP1A1-mediated oxidation of carcinogenic azo dye Sudan I and its binding to DNA by cytochrome b5. Neuro Endocrinol Lett 27(Suppl 2):31–34.

Stiborová M, Martínek V, Semanská M, Hodek P, Dračínský M, Cvačka J, Schmeiser HH, Frei E. 2009. Oxidation of the carcinogenic non-aminoazo dye 1-phenylazo-2-hydroxy-naphthalene (Sudan I) by cytochromes P450 and peroxidases: a comparative study. Interdiscip Toxicol 2:195–200.

Stiborová M, Dračínská H, Martínek V, Svášková D, Hodek P, Milichovský J, Hejduková Z, Brotánek J, Schmeiser HH, Frei E. 2013. Induced expression of cytochrome P450 1A and NAD(P)H:quinone oxidoreductase determined at mRNA, protein, and enzyme activity levels in rats exposed to the carcinogenic azo dye 1-phenylazo-2-naphthol (Sudan I). Chem Res Toxicol 26:290–299.

Stoner GD, Conran PB, Greisiger EA, Stober J, Morgan M, Pereira MA. 1986. Comparison of two routes of chemical administration on the lung adenoma response in strain A/J mice. Toxicol Appl Pharmacol 82:19–31.

Storer RD, McKelvey TW, Kraynak AR, Elia MC, Barnum JE, Harmon LS, Nichols WW, DeLuca JG. 1996. Revalidation of the *in vitro* alkaline elution/rat hepatocyte assay for DNA damage: improved criteria for assessment of cytotoxicity and genotoxicity and results for 81 compounds. Mutat Res 368:59–101.

Suter W, Jaeger I. 1982. Comparative evaluation of different pairs of DNA repair-deficient and DNA repair-proficient bacterial tester strains for rapid detection of chemical mutagens and carcinogens. Mutat Res 97:1–18.

Suzuki H, Ikeda N, Kobayashi K, Terashima Y, Shimada Y, Suzuki T, Hagiwara T, Hatakeyama S, Nagaoka K, Yoshida J. 2005. Evaluation of liver and peripheral blood micronucleus assays with 9 chemicals using young rats. A study by the Collaborative Study Group for the Micronucleus Test (CSGMT)/Japanese Environmental Mutagen Society (JEMS)-Mammalian Mutagenicity Study Group (MMS). Mutat Res 583:133–145.

Suzuki H, Komatsu K, Imamura T, Miyazaki A, Kobayashi T, Nomura M. 2006. Genotoxicity studies of *p*-dimethylaminoazobenzene (DAB). J Toxicol Sci 31:399–405.

Suzuki H, Takasawa H, Kobayashi K, Terashima Y, Shimada Y, Ogawa I, Tanaka J, Imamura T, Miyazaki A, Hayashi M. 2009. Evaluation of a liver micronucleus assay with 12 chemicals using young rats (II): a study by the Collaborative Study Group for the Micronucleus Test/Japanese Environmental Mutagen Society–Mammalian Mutagenicity Study Group. Mutagenesis 24:9–16.

Szybalski W. 1958. Special microbiological systems. 2. Observations on chemical mutagenesis in microorganisms. Ann NY Acad Sci 76:475–489.

Takagishi T, Katayama A, Konishi K, Kuroki N. 1969. The solubilities of azobenzene derivatives in water. Kolloid. Zeitschrift und Zeitschrift fur Polymere, Band 232, Heft 1, p. 693–699.

Takasawa H, Suzuki H, Ogawa I, Shimada Y, Kobayashi K, Terashima Y, Matsumoto H, Oshida K, Ohta R, Imamura T, Miyazaki A, Kawabata M, Minowa S, Maeda A, Hayashi M. 2010. Evaluation of a liver micronucleus assay in young rats (IV): a study using a double-dosing/single-sampling method by the Collaborative Study Group for the Micronucleus Test (CSGMT)/Japanese Environmental Mutagen Society (JEMS)–Mammalian Mutagenicity Study Group (MMS). Mutat Res 698:24–29.

Takayama S, Thorgeirsson UP, Adamson RH. 2008. Chemical carcinogenesis studies in nonhuman primates. Proc Jpn Acad Ser B Phys Biol Sci 84(6):176–188.

Tennant RW, Margolin BH, Shelby MD, Zeiger E, Haseman JK, Spalding J, Caspary W, Resnick M, Stasiewicz S, Anderson B, Minor R. 1987a. Prediction of chemical carcinogenicity in rodents from *in vitro* genetic toxicity assays. Science 236:933–941.

Tennant RW, Spalding JW, Stasiewicz S, Caspary WD, Mason JM, Resnick MA. 1987b. Comparative evaluation of genetic toxicity patterns of carcinogens and noncarcinogens: strategies for predictive use of short-term assays. Environ Health Perspect 75:87–95.

Terracini B, Della Porta G. 1961. Feeding with aminoazo dyes, thioacetamide, and ethionine. Studies in the hamster. Arch Pathol 71:566–575.

[Therapeutic Guidelines] Therapeutic Guidelines Limited. 2008. Lund and Browder chart for calculating the percentage of total body surface area burnt (Figure 14.19). Published in eTG complete, March 2008. [Internet]. Available upon request.

Timofeeva OA, Eremeev AV, Goloshchapov A, Kalashnikova E, Ilnitskaya S, Setkov NA, Kobzev V, Buzard GS, Filipenko ML, Kaledin VI, Merkulova TI. 2008. Effects of o-aminoazotoluene on liver regeneration and p53 activation in mice susceptible and resistant to hepatocarcinogenesis. Toxicology 254:91-6.

Tincher WC, Robertson JR. 1982. Analysis of dyes in textile dyeing wastewater. Text Chem Color 14:269–275. [cited in IARC 1990].

Tomatis L, Della Porta G, Shubik P.1961. Urinary bladder and liver cell tumors induced in hamsters with *o*-aminoazotoluene. Cancer Res 21:1513–1517.

Tong C, Fazio M, Telang S, Williams GM. 1980. The detection of genotoxic chemicals in the adult rat liver epithelial cell/hypoxanthine-guanine phosphoribosyl transferase (ARL/HGPRT) mutagenesis assay. Ann NY Acad Sci 349:407–408.

Tong C, Telang S, Williams GM. 1984. Differences in responses of 4 adult rat-liver epithelial cell lines to a spectrum of chemical mutagens. Mutat Res 130:53–62.

Tonogai Y, Ogawa S, Ito Y, Iwaida M. 1982. Actual survey on TL_m (median tolerance limit) values of environmental pollutants, especially on amines, nitriles, aromatic nitrogen compounds and artificial dyes. J Toxicol Sci 7:193–203.

Topham JC. 1980. Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens? Mutat Res 74:379–387.

Tosato ML, Pino A, Passerini L, Marchini S, Vigano L, Hoglund MD. 1993. Updating and validation of a *Daphnia* toxicity model for benzene derivatives. Sci Total Environ 134(Suppl 2):1479–1490.

Trattner A, Farchi Y, David M. 2003. Shoe contact dermatitis in Israel. Am J Contact Dermatitis 14:12–14.

Tsuchimoto T, Matter BE. 1981. Activity of coded compounds in the micronucleus test. Prog Mutat Res 1:705–711.

Tsuchiya T, Ikarashi Y, Nakamura A. 1995. Studies on the tumor-promoting activities of additives in biomaterials: inhibition of metabolic cooperation by additives such as pigments and phenolic antioxidants. J Long Term Eff Med Implants 5:243–252.

Tsuda S, Matsusaka N, Madarame H, Ueno S, Susa N, Ishida K, Kawamura N, Sekihashi K, Sasaki YF. 2000. The comet assay in eight mouse organs: results with 24 azo compounds. Mutat Res 465:11–26.

Tsuda S, Murakami M, Matsusaka N, Kano K, Taniguchi K, Sasaki YF. 2001. DNA damage induced by red food dyes orally administered to pregnant and male mice. Toxicol Sci 61:92–99.

Tucker JD, Auletta A, Cimino MC, Dearfield KL, Jacobson-Kram D, Tice RR, Carrano AV. 1993. Sister-chromatid exchange: second report of the Gene-Tox Program. Mutat Res 297:101–180.

Urushigawa Y, Yonezawa Y. 1977. Chemico-biological interactions in biological purification system II. Biodegradation of azocompounds by activated sludge. Bull Environ Contam Tox 17(29):214–218.

[US EPA] US Environmental Protection Agency. 1993. Azobenzene (CASRN 103-33-3) [Internet]. Washington (DC): US EPA, Integrated Risk Information System (IRIS). Available from: www.epa.gov/iris/subst/0351.htm

[US EPA] US Environmental Protection Agency. 2011. Exposure factors handbook: 2011 edition. Washington (DC): US EPA, Office of Research and Development, National Center for Environmental Assessment. Report No.: EPA/600/R-09/052F. Available from: www.epa.gov/ncea/efh/pdfs/efh-complete.pdf

[US EPA] US Environmental Protection Agency. 2012. Standard operating procedures for residential pesticide exposure assessment. Washington (DC): US EPA, Office of Chemical Safety and Pollution Prevention, Office of Pesticide Programs, Health Effects Division. Available from: www.epa.gov/pesticides/science/EPA-OPP-HED_Residential%20SOPS_Feb2012.pdf

[US EPA] US Environmental Protection Agency. 2013. Benchmark Dose Software (BMDS version 2.3.1). Available from: www.epa.gov/ncea/bmds/

[US NRC] US National Research Council. 1999. Toxicity of military smokes and obscurants, vol. 3. Washington (DC): US NRC, Subcommittee on Military Smoke and Obscurants. Available from: http://search.nap.edu/nap-cgi/skimchap.cgi?recid=9645&chap=74-78

Uter W, Geier J, Lessmann H, Hausen BM. 2001. Contact allergy due to Disperse Blue 106 and Disperse Blue 124 in German and Austrian patients, 1995 to 1999. Contact Dermatitis 44:173–177.

Uter W, Lessmann H, Geier J, Becker D, Fuchs T, Richter G, IVDK Study Group, German Contact Dermatitis Research Group (DKG). 2002. The spectrum of allergic (cross-)sensitivity in clinical patch testing with “para amino” compounds. Allergy 57:319–322.

Valks R, Conde-Salazar L, Malfeito J, Ledo S. 2005. Contact dermatitis in hairdressers, 10 years later: patch-test results in 300 hairdressers (1994 to 2003) and comparison with previous study. Dermatitis 16:28–31.

Van Hoogen GJ, Opperhuizen A. 1988. Toxicokinetics of chlorobenzenes in fish. Environ Toxicol Chem 7:213–219.

Vogel EW, Nivard MJ. 1993. Performance of 181 chemicals in a *Drosophila* assay predominantly monitoring interchromosomal mitotic recombination. Mutagenesis 8:57–81.

Wakata A, Miyamae Y, Sato S, Suzuki T, Morita T, Asano N, Awogi T, Kondo K, Hayashi M. 1998. Evaluation of the rat micronucleus test with bone marrow and peripheral blood: summary of the 9th collaborative study by CSGMT/JEMS.MMS. Environ Mol Mutagen 32:84–100.

Walker AIT, Thorpe E, Stevenson DE. 1972. I. Long-term oral toxicity studies in mice. Food Cosmet Toxicol 11:415–432.

Wantke F, Gotz M, Jarisch R. 1992. Contact dermatitis due to henna, Solvent Red 1 and Solvent Red 3. A case report. Contact Dermatitis 27:346–347.

Washington State Department of Ecology. 2014. *Children's Safe Product Act* reports [Internet]. [cited 2014 Aug 18]. Available from: https://fortress.wa.gov/ecy/cspareporting/

Watanabe HK, Hashimoto Y. 1981. Unscheduled DNA synthesis induced by 4-aminoazobenzene, *N*-hydroxy-4-aminoazobenzene, and their derivatives in primary cultures of rat and mouse hepatocytes. Gann 72:930–936.

Watanabe M, Satake K, Kurogochi S. 1999. Evaluation of micronucleus induction in rats by Oil Orange SS. Kankyo Hen'Igen Kenkyu 21(3):251–253.

Waterman N, Lignac G. 1958. The influence of the feeding of a number of food colours on the occurrence of tumours in mice. Acta Physiol Pharmacol Neerl 7(1):35–55.

Waters LL. 1937. *O*-Aminoazotoluene as a carcinogenic agent. Yale J Biol Med 10:179–185.

Watson E. 2007. Market share and company ranking [Internet]. Toronto (ON): York University, York University Libraries. [created 2007 Apr; revised 2010 Jan; cited 2010 Aug 2]. Available from: www.library.yorku.ca/cms/bbl/assignments/marketshare/

Weber EJ, Wolfe NL. 1987. Kinetic studies of the reduction of aromatic azo compounds inanaerobic sediment/water systems. Environ Toxicol Chem 6:911–919.

Weber EJ, Alizzacolon D, Baughman GL. 2001. Sediment-associated reactions of aromatic amines. 1. Elucidation of sorption mechanisms. Environ Sci Technol 35:2470–2475.

Westmoreland C, Gatehouse DG. 1991. The differential clastogenicity of Solvent Yellow 14 and FD & C Yellow No. 6 *in vivo* in the rodent micronucleus test (observations on species and tissue specificity). Carcinogenesis 12:1403–1408.

Weyman GS, Rufli H, Weltje L, Salinas ER, Hamitou M. 2012. Aquatic toxicity tests with substances that are poorly soluble in water and consequences for environmental risk assessment. Environ Toxicol Chem 31(7):1662–1669.

Wilkinson SM, Thomson KF. 2000. Foot dermatitis due to non-disperse azo dyes. Contact Dermatitis 42:162–163.

Willheim R, Ivy AC. 1953. A preliminary study concerning the possibility of dietary carcinogenesis. Gastroenterology 23:1–19.

Williams GM. 1976. The use of liver epithelial cultures for the study of chemical carcinogenesis. Am J Pathol 85:739–753.

Williams GM. 1977. Detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell cultures. Cancer Res 37:1845–1851.

Williams JH. 1999. Regulations on additions of sludge-borne metals to soil and their adaptation to local conditions. In: L'Hermite P, editor. Treatment and use of sewage sludge and liquid agricultural wastes. London (GB): Commission of the European Communities; Elsevier Applied Science; p. 243–250.

Wisniewska-Knypl JM. 1978. Activity of drug-metabolizing microsomal enzymes in rats exposed to aniline, azobenzene and drugs of benzodiazepine group. In: Fouts JR, Crut I, editors. Industrial and environmental xenobiotics. (International Congress Series No. 440). Amsterdam (NL): Excerpta Medica; p. 141–144.

Wormuth M, Scheringer M, Hungerbühler K. 2005. Linking the use of scented consumer products to consumer exposure to polycyclic musk fragrances. J Ind Ecol 9(1–2):237–258.

[WSKOWWIN] Water Solubility for Organic Compounds Program for Microsoft Windows [Estimation Model]. 2010. Version 1.42. Washington (DC): US Environmental Protection Agency, Office of Pollution Prevention and Toxics; Syracuse (NY): Syracuse Research Corporation. Available from: www.epa.gov/oppt/exposure/pubs/episuite.htm

Wu X, Bennett DH, Ritz B, Cassady DL, Lee K, Hertz-Picciotto I. 2010. Usage pattern of personal care products in California households. Food Chem Toxicol 48: 3109-3119.

Wyrobek AJ, Gordon L, Watchmaker G. 1981. Effect of 17 chemical agents including 6 carcinogen/noncarcinogen pairs of sperm shape abnormalities in mice. Prog Mutat Res 1:712–717.

Wyrobek AJ, Gordon LA, Burkhart JG, Francis MW, Kapp RW Jr, Letz G, Malling HV, Topham JC, Whorton MD. 1983. An evaluation of the mouse sperm morphology test and other sperm tests in nonhuman mammals: a report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat Res 115:1–72.

Xie S, Gu Z, Zhou D. 1999. [Sensitivity of photobacterial dark mutant for detecting chemical mutagenicity]. Huanjing Kexue Xuebao 19(3):313–318. [in Chinese].

Xie Z, Hayakawa R, Sugiura M, Kojima H, Konishi H, Ichihara G, Takeuchi Y. 2000. Experimental study on skin sensitization potencies and cross-reactivities of hair-dye-related chemicals in guinea pigs. Contact Dermatitis 42:270–275.

Xu H, Heinze TM, Chen S, Cerniglia CE, Chen H. 2007. Anaerobic metabolism of 1-amino-2-naphthol-based azo dyes (Sudan dyes) by human intestinal microflora. Appl Environ Microbiol 73:7759–7762.

Xu H, Heinze TM, Paine DD, Cerniglia CE, Chen H. 2010. Sudan azo dyes and para red degradation by prevalent bacteria of the human gastrointestinal tract. Anaerobe 16:114–119.

Yahagi T, Degawa M, Seino Y, Matsushima T, Nagao M. 1975. Mutagenicity of carcinogenic azo dyes and their derivatives. Cancer Lett 1:91–96.

Yalkowsky SH, Dannenfelser RM. 1992. Aquasol database of aqueous solubility. Version 5. College of Pharmacy. University of Arizona-Tucson, AZ. PC Version.

Yamazaki H, Oda Y, Shimada T. 1992. Use of a newly developed tester strain *Salmonella typhimurium* NM2009 for the study of metabolic activation of carcinogenic aromatic amines by rat liver microsomal cytochrome P-450 enzymes. Mutat Res 272:183–192.

Yasunaga K, Kiyonari A, Oikawa T, Abe N, Yoshikawa K. 2004. Evaluation of the *Salmonella* umu test with 83 NTP chemicals. Environ Mol Mutagen 44:329–345.

Yen CC, Perenich TA, Baughman GL. 1991. Fate of commercial disperse dyes in sediments. Environ Toxicol Chem 10:1009–1017.

Zeiger E. 1987. Carcinogenicity of mutagens: predictive capability of the *Salmonella* mutagenesis assay for rodent carcinogenicity. Cancer Res 47:1287–1296.

Zeiger E, Anderson B, Haworth S. 1987. *Salmonella* mutagenicity tests: III. Results from the testing of 255 chemicals. Environ Mutagen 9(Suppl 9):1–110.

Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. 1988. *Salmonella* mutagenicity tests: IV. Results from the testing of 300 chemicals. Environ Mol Mutagen 11(Suppl 12):1–157.

Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. 1992. *Salmonella* mutagenicity tests: V. Results from the testing of 311 chemicals. Environ Mol Mutagen 19(Suppl 21):2–141.

Zeilmaker MJ, Kroese ED, van Haperen P, van Veen MP, Bremmer HJ, van Kranen HJ, Wouters MFA, Janus JA. 1999. Cancer risk assessment of azo dyes and aromatic amines from garment and footwear. Bilthoven (NL): Rijkinstituut voor Volkgezondheid en Milieu (National Institute of Public Health and the Environment). RIVM Report No.: 601503 014. Available from: www.rivm.nl/bibliotheek/rapporten/601503014.pdf

Zeilmaker MJ, van Kranen HJ, van Veen MP, Janus JA. 2000. Cancer risk assessment of azo dyes and aromatic amines from tattoo bands, folders of paper, toys, bed clothes, watch straps and ink. Bilthoven (NL): Rijksinstituut voor Volksgezondheid en Milieu (National Institute of Public Health and the Environment); 45 p. RIVM Report No.: 601503 019. Available from: www.rivm.nl/bibliotheek/rapporten/601503019.pdf

Zhang X, Jiang L, Geng C, Hu C, Yoshimura H, Zhong L. 2008. Inhibition of Sudan I genotoxicity in human liver-derived HepG2 cells by the antioxidant hydroxytyrosol. Free Radic Res 42:189–195.

Zhou Y, You X, Ye X. 1987. [Mutagenicity study for aminoazobenzene and its derivatives]. Huanjing Kexue 8(2):31–34. [in Chinese].

Zimmermann FK, von Borstel RC, von Halle ES. 1984. Testing of chemicals for genetic activity with *Saccharomyces cerevisiae*: a report of the US Environmental Protection Agency Gene-Tox Program. Mutat Res 133:199–244.

Zimmering S, Mason JM, Valencia R, Woodruff RC. 1985. Chemical mutagenesis testing in *Drosophila*. II. Results of 20 coded compounds tested for the National Toxicology Program. Environ Mutagen 7:87–100.

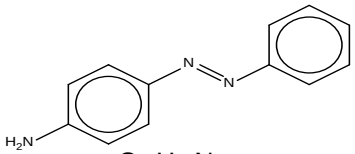
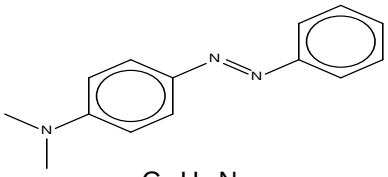
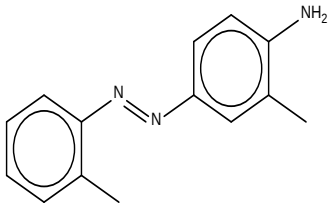
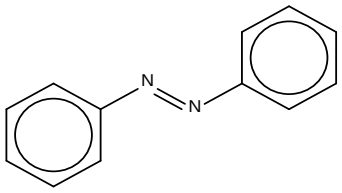
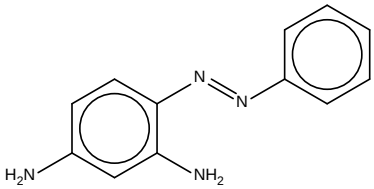
Zina G, Bonu G. 1965. [Role of azo dyes as primary contact allergen]. Minerva Dermatol 40:307–314. [in Italian].

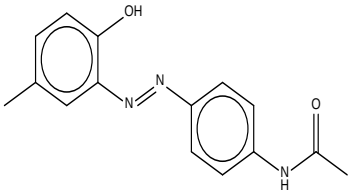
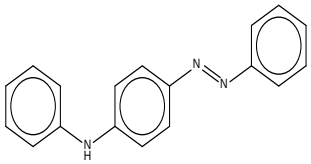
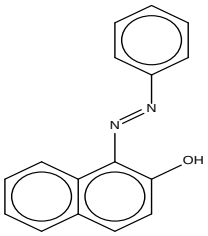
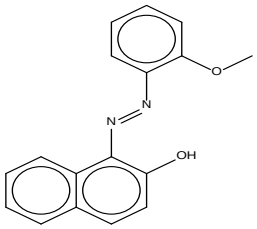
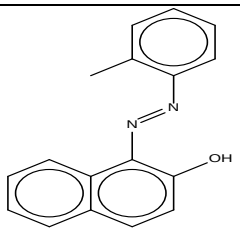
Zoeteman BCJ, Harmsen K, Linders JBHJ, Morra CFH, Slooff W. 1980. Persistent organic pollutants in river water and groundwater of the Netherlands. Chemosphere 9:231–249.

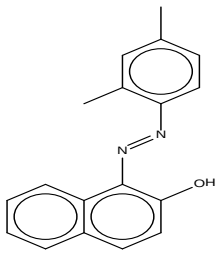
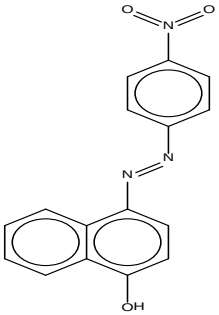
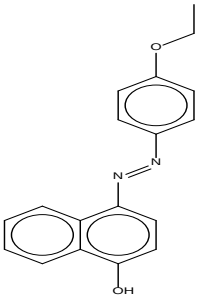
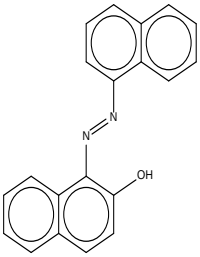
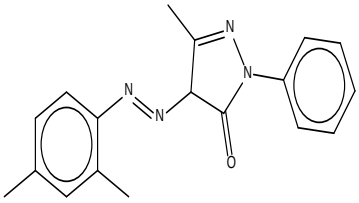
Appendices

Appendix A: Structural Identity of Azo Solvent Dyes and Analogues

Table A-1: Structural identity, eco-subset information and health subset information for the individual monoazo solvent dyes

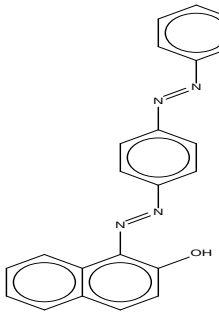
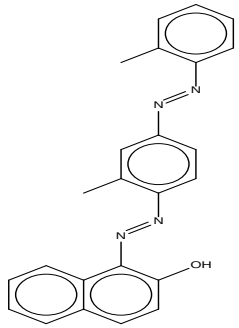
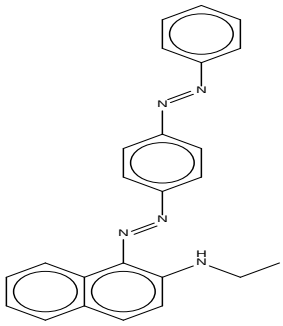
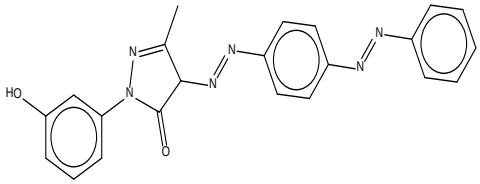
CAS RN and eco-subset	C.I. name or common name	Chemical structure and chemical formula	Molecular weight (g/mol)	Health subset
60-09-3 (A)	Solvent Yellow 1 or *p*-aminoazobenzene	 $C_{12}H_{11}N_3$	197.2	Azobenzene and Its Derivatives
60-11-7 (A)	Solvent Yellow 2	 $C_{14}H_{15}N_3$	225.3	Azobenzene and Its Derivatives
97-56-3 (A)	Solvent Yellow 3	 $C_{14}H_{15}N_3$	225.3	Azobenzene and Its Derivatives
103-33-3 (A)	Azobenzene	 $C_{12}H_{10}N_2$	182.2	Azobenzene and Its Derivatives
495-54-5 (A)	Solvent Orange 3	 $C_{12}H_{12}N_4$	212.3	Azobenzene and Its Derivatives

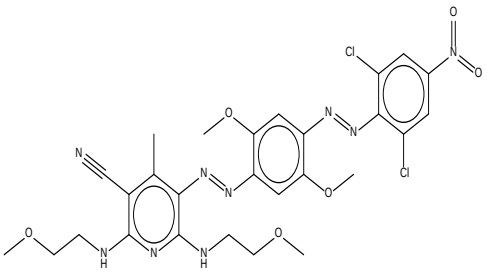
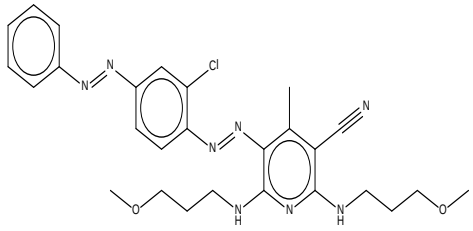
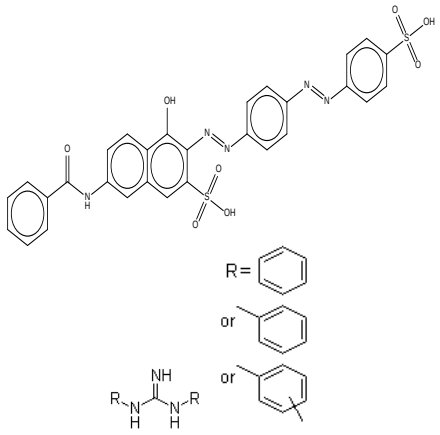
CAS RN and eco-subset	C.I. name or common name	Chemical structure and chemical formula	Molecular weight (g/mol)	Health subset
2832-40-8 (A)	Solvent Yellow 77	 $C_{15}H_{15}N_3O_2$	269.3	Azobenzene and Its Derivatives
101-75-7 (B)	4-Anilinoazobenzene	 $C_{18}H_{15}N_3$	273.3	Miscellaneous Substances
842-07-9 (C)	Solvent Yellow 14 or Sudan I	 $C_{16}H_{12}N_2O$	248.3	Sudan Dyes
1229-55-6 (C)	Solvent Red 1	 $C_{17}H_{14}N_2O_2$	278.3	Sudan Dyes
2646-17-5 (C)	Solvent Orange 2 or Oil Orange SS	 $C_{17}H_{14}N_2O$	262.3	Sudan Dyes

CAS RN and eco-subset	C.I. name or common name	Chemical structure and chemical formula	Molecular weight (g/mol)	Health subset
3118-97-6 (C)	Solvent Orange 7	 $C_{18}H_{16}N_2O$	276.3	Sudan Dyes
5290-62-0 (C)	Magneson II	 $C_{16}H_{11}N_3O_3$	293.3	Miscellaneous Substances
6535-42-8 (C)	Solvent Red 3	 $C_{18}H_{16}N_2O_2$	292.3	Miscellaneous Substances
2653-64-7 (C)	Solvent Red 4	 $C_{20}H_{14}N_2O$	298.3	Miscellaneous Substances
6407-78-9 (D)	Solvent Yellow 18	 $C_{18}H_{18}N_4O$	306.4	Miscellaneous Substances

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

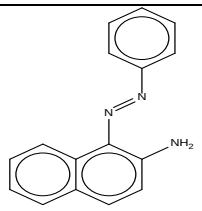
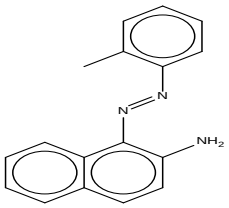
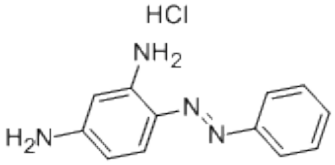
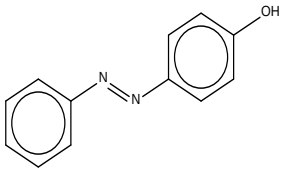
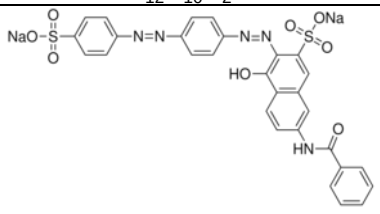
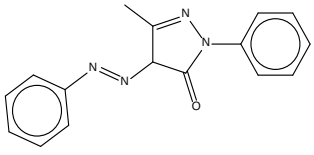
Table A-2: Structural identity, eco-subset information and health subset information for the individual disazo solvent dyes

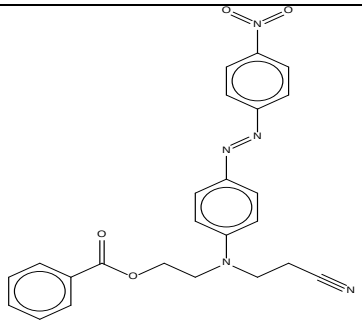
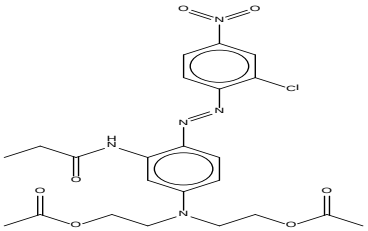
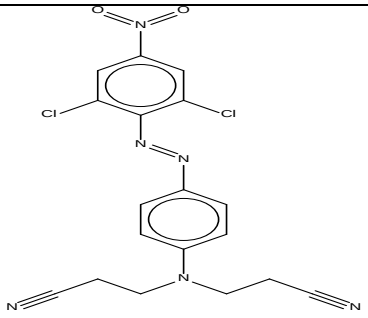
CAS RN and eco-subset	C.I. name or common name	Chemical structure and chemical formula	Molecular weight (g/ mol)	Health subset
85-86-9 (E)	Solvent Red 23	 $C_{22}H_{16}N_4O$	352.4	Sudan Dyes
85-83-6 (E)	Solvent Red 24 or Sudan IV	 $C_{24}H_{20}N_4O$	380.5	Sudan Dyes
6368-72-5 (E)	Solvent Red 19	 $C_{24}H_{21}N_5$	379.5	Miscellaneous Substances
21519-06-2 (F)	NA	 $C_{22}H_{18}N_6O_2$	398.4	Miscellaneous Substances

CAS RN and eco-subset	C.I. name or common name	Chemical structure and chemical formula	Molecular weight (g/ mol)	Health subset
73528-78-6 (G)	NA	 $C_{27}H_{29}Cl_2N_9O_6$	646.5	Miscellaneous Substances
85392-21-8 (G)	NA	 $C_{27}H_{31}ClN_8O_2$	535.1	Miscellaneous Substances
73507-36-5 (H)	NA (UVCB)	 $C_{29}H_{22}N_5O_8$	568.5	Miscellaneous Substances

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index; NA, not available; UVCB, unknown or variable composition, complex reaction products, or biological materials

Table A-3: Structural identity information for analogues used to inform the ecological and human health assessments of the Azo Solvent Dyes

CAS RN and subset	Chemical name (C.I. name or common name)	Chemical structure and chemical formula	Molecular weight (g/mol)
85-84-7 (C)	1-(Phenylazo)-2-naphthylamine (Solvent Yellow 5 or Oil Yellow AB)	 $C_{16}H_{13}N_3$	247.3
131-79-3 (C)	1-((2-Methylphenyl)azo)-2-naphthalenamine (Solvent Yellow 6 or Oil Yellow OB)	 $C_{17}H_{15}N_3$	261.4
532-82-1 (human health)	1,3-Benzenediamine, 4-(phenylazo), monohydrochloride (Basic Orange 2)	 $C_{12}H_{12}N_4$-HCl	248.7
1689-82-3 (A)	4-Phenylazophenol (Solvent Yellow 7)	 $C_{12}H_{10}N_2O$	198.2
2610-11-9 (H)	2-Naphthalenesulfonicacid,7-(benzoylamino)-4-hydroxy-3-[2-[4-[2-(4-sulfophenyl)diazenyl]phenyl]diazenyl]-, sodium salt (1:2) (Direct Red 81)	 $C_{29}H_{19}N_5Na_2O_8S_2$	675.6
4314-14-1 (human health)	3H-Pyrazol-3-one,2,4-dihydro-5-methyl-2-phenyl-4-(2-phenyldiazenyl)- (Solvent Yellow 16 or Sudan Yellow 3G)	 $C_{16}H_{14}N_4O$	278.3

CAS RN and subset	Chemical name (C.I. name or common name)	Chemical structure and chemical formula	Molecular weight (g/mol)
40690-89-9 (E)	Propanenitrile, 3-((2-(benzoyloxy)ethyl)(4-((4-nitrophenyl)azo)phenyl) amino)- (Disperse Orange 73)	 $C_{24}H_{21}N_5O_4$	443.5
61968-52-3 (G)	Propanamide, *N*-[5-[bis[2-(acetyloxy)ethyl]amino]-2-[2-(2-chloro-4-nitrophenyl)diazenyl]phenyl]- (Disperse Red 167)	 $C_{23}H_{26}ClN_5O_7$	520.0
71767-67-4 (G)	Propanenitrile, 3,3′-[[4-[2-(2,6-dichloro-4-nitrophenyl)diazenyl]phenyl]imino]bis- (Disperse Yellow 163)	 $C_{18}H_{14}Cl_2N_6O_2$	417.3

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

Appendix B. Physical and Chemical Properties of the Azo Solvent Dyes and Analogues

Table B-1: Physical and chemical properties of the individual monoazo solvent dyes with experimental data

CAS RN, C.I. name (eco-subset)	Property	Value	Reference
60-09-3 *p*-Aminoazobenzene (A)	Physical state	Brownish-yellow powder	ChemicalBook 2008
	Melting point (°C)	128	Merck Index 2006
		125	Janado et al. 1980
	Vapour pressure (Pa)	1.87×10^{-4} (extrapolated)	Shimizu et al. 1987
	Water solubility (mg/L)	32 (at 25°C)	Shibusawa et al. 1977
		34.3 (at 25°C)	Takagishi et al. 1969
		127 (at 18°C)	Pfeiffer 1925
		Slightly soluble	HSDB 1983–
	Other solubilities	Soluble in ethanol, benzene, chloroform, ether	HSDB 1983–
	Log K_{ow}	3.41	Hansch et al. 1995
		2.62	Leo et al. 1971
		1.49	Tonogai et al. 1982
60-11-7 Solvent Yellow 2 (A)	Physical state	Yellow-orange crystalline powder	ChemicalBook 2008
	Melting point (°C)	114–117	Merck Index 2006
		117	Bird 1954
		116	Takagishi et al. 1969
		123	Green and Jones 1967
		111	CAMEO Chemicals ©2011
	Vapour pressure (Pa)	9.33×10^{-6} (extrapolated)	Campanelli et al. 1985
	Water solubility (mg/L)	0.23	Baughman and Perenich 1988a
		< 1	CAMEO Chemicals ©2011
		1.4 (at 25°C)	Janado et al. 1980
		0.38 (at 25°C)	Takagishi et al. 1969
		0.23 (at 25°C)	Bird 1954
		Insoluble	HSDB 1983–
	Other solubilities	Soluble in ethanol, benzene, chloroform, ether, petroleum ether, mineral acids, oils	HSDB 1983–
	Log K_{ow}	2.03	Tonogai et al. 1982
		4.58	Hansch et al. 1995; Radding et al. 1977; Perrin 1972
		4.05	Takagishi et al. 1969
	pK_a	3.23	Perrin 1972
97-56-3 Solvent Yellow 3 (A)	Physical state	Red-brown crystalline powder	ChemicalBook 2008
	Melting point (°C)	101–102	Merck Index 2006; Sigma-Aldrich 2010; Acros Organics N.V. 2008a
		102	Yalkowsky and Dannenfelser 1992
	Vapour pressure	10.0×10^{-5} (extrapolated)	Shimizu et al. 1987

CAS RN, C.I. name (eco-subset)	Property	Value	Reference
	(Pa)		
	Henry's law constant ($Pa \cdot m^3/mol$)	0.003 22 (extrapolated)	OECD QSAR Toolbox 2012
	Water solubility (mg/L)	7	Yalkowsky & Dannenfelser 1992
		0.1	Green 1990
		Practically insoluble	HSDB 1983–
	Other solubilities	Soluble in ethanol, ether, chloroform, acetone, cellosolve, toluene	HSDB 1983–
	Log K_{ow}	4.25	Radding et al. 1977
103-33-3 Azobenzene (A)	Physical state	Orange to red crystals	ChemicalBook 2008
	Melting point (°C)	68	Takagishi et al. 1969, Lewis and Sax 2000, Merck Index 2006
		67.88	Lide 2005–2006
	Vapour pressure (Pa)	0.0481	Jones 1960
	Henry's law constant ($Pa \cdot m^3/mol$)	1.37 (extrapolation)	OECD QSAR Toolbox 2012
	Water solubility (mg/L)	6.4 (at 25°C)	Takagishi et al. 1969
		0.29–8.37	Janado et al. 1980
		< 0.1	CAMEO Chemicals ©2011
		Insoluble	HSDB 1983–
	Other solubilities	Soluble in ethanol, ether, glacial acetic acid	HSDB 1983–
	Log K_{ow}	3.82	Hansch et al. 1995
		1.56	Tonogai et al. 1982
		3.13	Briggs 1981
	pK_a	−2.48	Perrin 1972
495-54-5 Solvent Orange 3 (A)	Physical state	Black liquid	LookChem ©2008
	Melting point (°C)	118–118.5	Lewis and Sax 2000
	Log K_{ow}	1.72	Tonogai et al. 1982
2832-40-8 Solvent Yellow 77 (A)	Physical state	Brownish-yellow powder	ChemicalBook 2008
	Melting point (°C)	195	Patterson and Sheldon 1960
		191.0–192.2	Nishida et al. 1989
	Vapour pressure (Pa)	6.67×10^{-9} (extrapolated)	Baughman and Perenich 1988b
	Henry's law constant ($Pa \cdot m^3/mol$)	1.52×10^{-6} (extrapolated)	OECD QSAR Toolbox 2012
	Water solubility (mg/L)	1.5–6.1 (at 60°C)	Patterson and Sheldon 1960
		1–2 (at 25°C)	Bird 1954
		1.18	Baughman and Perenich 1988b
		20	Green 1990
	Other solubilities	Soluble in acetone, ethanol,	HSDB 1983–

CAS RN, C.I. name (eco-subset)	Property	Value	Reference
		benzene	
	Log K_{ow}	3.6	Sigma-Aldrich 2010
101-75-7 4-Anilinoazobenzene (B)	Physical state	Orange fine crystalline powder	ChemicalBook 2008
	Melting point (°C)	89–91	Meylan et al. 1996
	Water solubility (mg/L)	< 0.1	Green 1990
	pK_a	0.99, 1.55	Perrin 1972
842-07-9 Sudan I (C)	Physical state	Orange red powder	ChemicalBook 2008
	Melting point (°C)	134	Meylan et al. 1996
		131–133	Green 1990
	Water solubility (mg/L)	0.5	Green 1990
		Insoluble	HSDB 1983–
	Other solubilities	Soluble in ethanol, acetone, ether, benzene	HSDB 1983–
1229-55-6 Solvent Red 1 (C)	Physical state	Red powder	Guidechem ©2010–2013
	Melting point (°C)	183	Hou et al. 1991
	Water solubility (mg/L)	3.3×10^{-4}	Baughman and Weber 1991
		0.0078	Balakrishnan 2013
	Log K_{ow}	7.5	Hou et al. 1991
2646-17-5 Oil Orange SS (C)	Physical state	Red powder	ChemicalBook 2008
	Melting point (°C)	132–133	Buckingham 1982
		131	HSDB 1983–
	Water solubility (mg/L)	Insoluble	HSDB 1983–
		Insoluble	HSDB 1983–
	Other solubilities	Slightly soluble in ethanol, chloroform, benzene	HSDB 1983–
3118-97-6 Solvent Orange 7 (C)	Physical state	Red to orange-brownish powder	LookChem ©2008
	Melting point (˚C)	166	Meylan et al. 1996
	Water solubility (mg/L)	8	Green 1990
		Insoluble	HSDB 1983–
	Other solubilities	Soluble in ethanol, acetone, benzene, ether	HSDB 1983–
5290-62-0 Magneson II (C)	Physical state	Red to brown powder	Acros Organics N.V. 2008b
	Melting point (°C)	270	Acros Organics N.V. 2008b; Alfa Aesar ©2011
6535-42-8 Solvent Red 3 (C)	Physical state	Solid	MP Biomedicals, LLC. 2006
	Melting point (°C)	152–155	MP Biomedicals, LLC. 2006
	Water solubility (mg/L)	0.3	Green 1990
		< 1	Clariant 2008
2653-64-7 Solvent Red 4 (C)	Density	1.2 g/cm^3	ChemNet 2013
	Vapour pressure (Pa)	5.01×10^{-8}	
6407-78-9	NA	NA	NA

CAS RN, C.I. name (eco-subset)	Property	Value	Reference
Solvent Yellow 18 (D)			

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index; K_{ow}, octanol–water partition coefficient; NA, not available; pK_a, acid dissociation constant

Table B-2: Experimental physical and chemical properties of the individual disazo solvent dyes having experimental data

CAS RN, C.I. name (eco-subset)	Property	Value	Reference
85-83-6 Sudan IV (E)	Physical state	Reddish brown powder	ChemicalBook 2008
	Melting point (°C)	184–185	MITI 1992
	Water solubility (mg/L)	0.7	Green 1990
		Practically insoluble	PhysProp 2006
	Other solubilities	1 g dissolves in 15 mL chloroform; soluble in oils, fats, warm petroleum, paraffin, phenol; slightly soluble in acetone, ethanol, benzene	PhysProp 2006
85-86-9 Solvent Red 23 (E)	Physical state	Dark red to brown crystals or powder	ChemicalBook 2008
	Melting point (°C)	> 100	Aldon 2010
	Water solubility (mg/L)	0.0137	Balakrishnan 2013
		< 0.1	Green 1990
		Insoluble	PhysProp 2006
	Other solubilities	Soluble in chloroform, glacial acetic acid; moderately soluble in ethanol (3% at room temperature), ether, acetone, petrol ether, oils, waxes; very soluble in benzene	IARC 1975
6368-72-5 Solvent Red 19 (E)	Physical state	Dark red or purple powder	Accustandard 2008
	Melting point (°C)	130	Accustandard 2008; Sigma-Aldrich 2010
	Water solubility (mg/L)	0.7	Green 1990
		Practically insoluble	PhysProp 2006
	Other solubilities	Soluble in ethanol; very soluble in acetone, benzene	PhysProp 2006
21519-06-2 (F)	NA	NA	NA
73528-78-6 (G)	NA	NA	NA
85392-21-8 (G)	NA	NA	NA
73507-36-5 (H)	NA	NA	NA

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

Table B-3: Physical and chemical properties of the analogues used in the ecological assessment

CAS RN	Property	Value	Reference
85-84-7 Solvent Yellow 5 (C)	Physical state	Orange or red platelets	HSDB 1983–
	Melting point (°C)	102–104	HSDB 1983–
	Henry's Law constant ($Pa \cdot m^3/mol$)	NA	
	Water solubility (mg/L)	0.3 (at 37°C)	Yalkowsky and Dannenfelser 1992
	Other solubilities	Very soluble in ethanol, acetic acid; soluble in alcohol, carbon tetrachloride, vegetable oils	HSDB 1983–
	Log K_{ow}	NA	
	pK_a	NA	
131-79-3 Solvent Yellow 6 (C)	Physical state	Orange or yellow powder	HSDB 1983–
	Melting point (°C)	125–126	HSDB 1983–
	Vapour pressure (Pa)	3.35×10^{-7} (at 25°C)	Guidechem ©2010–2013
	Henry's Law constant ($Pa \cdot m^3/mol$)	NA	
	Water solubility (mg/L)	NA	
	Other solubilities	Soluble in alcohol, ether, benzene, carbon tetrachloride, vegetable oils, glacial acetic acid	HSDB 1983–
	Log K_{ow}	NA	
	pK_a	NA	
1689-82-3 Solvent Yellow 7 (A)	Physical state	Orange columnar solid	LookChem ©2008
	Melting point (°C)	155–157	HSDB 1983–
	Vapour pressure (Pa)	3.07×10^{-5}	Shimizu et al. 1987
	Henry's Law constant ($Pa \cdot m^3/mol$)	6.79×10^{-5}	Shimizu et al. 1987
	Water solubility (mg/L)	90 (at 20°C)	HSDB 1983– ; Yalkowsky and Dannenfelser 1992
	Other solubilities	Very soluble in acetone, ethanol, ether, benzene	HSDB 1983–
	Log K_{ow}	NA	
	pK_a	8.2	Perrin 1972
2610-11-9 Direct Red 81 (H)	Physical state	Solid	ReagentWorld Inc. 2013
	Melting point (°C)	240	ReagentWorld Inc. 2013
	Vapour pressure (Pa)	NA	
	Henry's Law constant ($Pa \cdot m^3/mol$)	NA	
	Water solubility (mg/L)	NA	
	Other solubilities	NA	
	Log K_{ow}	NA	
	pK_a	NA	

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index; K_{ow}, octanol–water partition coefficient; NA, not available; pK_a, acid dissociation constant

Table B-4. Modelled physical and chemical properties of the individual monoazo solvent dyes using EPI Suite (2012)

CAS RN, C.I. name (eco-subset)	Property	Value	Reference
60-09-3 *p*-Aminoazobenzene (A)	Melting point (°C)	93.58	MPBPWIN 2010
	Vapour pressure (Pa)	7.01×10^{-4}	MPBPWIN 2010
	Henry's Law constant ($Pa \cdot m^3/mol$)	5.27×10^{-4}	HENRYWIN 2011
	Water solubility (mg/L)	20.46	WSKOWWIN 2010
	Log K_{ow}	3.19	KOWWIN 2010
	Log K_{oc}	3.22	KOCWIN 2010
60-11-7 Solvent Yellow 2 (A)	Melting point (°C)	77.01	MPBPWIN 2010
	Vapour pressure (Pa)	4.96×10^{-3}	MPBPWIN 2010
	HLC ($Pa\text{-}m^3/mole$)	2.37×10^{-2}	HENRYWIN 2011
	Water solubility (mg/L)	1.463	WSKOWWIN 2010
	Log K_{ow}	4.29	KOWWIN 2010
	Log K_{oc}	3.82	KOCWIN 2010
97-56-3 Solvent Yellow 3 (A)	Melting point (°C)	116.21	MPBPWIN 2010
	Vapour pressure (Pa)	1.27×10^{-3}	MPBPWIN 2010
	Henry's Law constant ($Pa \cdot m^3/mol$)	6.41×10^{-4}	HENRYWIN 2011
	Water solubility (mg/L)	2.594	WSKOWWIN 2010
	Log K_{ow}	4.29	KOWWIN 2010
	Log K_{oc}	3.71	KOCWIN 2010
103-33-3 Azobenzene (A)	Melting point (°C)	13.57	MPBPWIN 2010
	Vapour pressure (Pa)	0.147	MPBPWIN 2010
	Henry's Law constant ($Pa \cdot m^3/mol$)	1.49	HENRYWIN 2011
	Water solubility (mg/L)	10.86	WSKOWWIN 2010
	Log K_{ow}	4.11	KOWWIN 2010
	Log K_{oc}	3.47	KOCWIN 2010
495-54-5 Solvent Orange 3 (A)	Melting point (°C)	132.77	MPBPWIN 2010
	Vapour pressure (Pa)	0	MPBPWIN 2010
	Henry's Law constant ($Pa \cdot m^3/mol$)	1.86×10^{-7}	HENRYWIN 2011
	Water solubility (mg/L)	213.4	WSKOWWIN 2010
	Log K_{ow}	2.13	KOWWIN 2010
	Log K_{oc}	2.49	KOCWIN 2010
2832-40-8 Solvent Yellow 77 (A)	Melting point (°C)	196.97	MPBPWIN 2010
	Vapour pressure (Pa)	6.35×10^{-8}	MPBPWIN 2010
	Henry's Law constant ($Pa \cdot m^3/mol$)	1.96×10^{-10}	HENRYWIN 2011
	Water solubility (mg/L)	10.25	WSKOW 2010
	Log K_{ow}	3.98	KOWWIN 2010
	Log K_{oc}	3.70	KOCWIN 2010
101-75-7 4-Anilinoazobenzene (B)	Melting point (°C)	137.1	MPBPWIN 2010
	Vapour pressure (Pa)	1.87×10^{-4}	MPBPWIN 2010
	Henry's Law constant ($Pa \cdot m^3/mol$)	2.91×10^{-4}	HENRYWIN 2011
	Water solubility (mg/L)	0.1553	WSKOWWIN 2010
	Log K_{ow}	5.41	KOWWIN 2010
	Log K_{oc}	4.31	KOCWIN 2010
842-07-9	Melting point (°C)	144.4	MPBPWIN 2010

CAS RN, C.I. name (eco-subset)	Property	Value	Reference
Sudan I (C)	Vapour pressure (Pa)	1.27×10^{-5}	MPBPWIN 2010
	Henry's Law constant ($Pa \cdot m^3/mol$)	1.51×10^{-5}	HENRYWIN 2011
	Water solubility (mg/L)	0.6738	WSKOWWIN 2010
	Log K_{ow}	5.51	KOWWIN 2010
	Log K_{oc}	4.57	KOCWIN 2010
1229-55-6 Solvent Red 1 (C)	Melting point (°C)	160.5	MPBPWIN 2010
	Vapour pressure (Pa)	8.39×10^{-7}	MPBPWIN 2010
	Henry's Law constant ($Pa \cdot m^3/mol$)	8.95×10^{-7}	HENRYWIN 2011
	Water solubility (mg/L)	0.3894	WSKOWWIN 2010
	Log K_{ow}	5.59	KOWWIN 2010
	Log K_{oc}	4.67	KOCWIN 2010
2646-17-5 Oil Orange SS (C)	Melting point (°C)	155.43	MPBPWIN 2010
	Vapour pressure (Pa)	3.63×10^{-6}	MPBPWIN 2010
	Henry's Law constant ($Pa \cdot m^3/mol$)	1.67×10^{-5}	HENRYWIN 2011
	Water solubility (mg/L)	0.1918	WSKOWWIN 2010
	Log K_{ow}	6.05	KOWWIN 2010
	Log K_{oc}	4.87	KOCWIN 2010
3118-97-6 Solvent Orange 7 (C)	Melting point (°C)	160.6	MPBPWIN 2010
	Vapour pressure (Pa)	1.35×10^{-6}	MPBPWIN 2010
	Henry's Law constant ($Pa \cdot m^3/mol$)	1.84×10^{-5}	HENRYWIN 2011
	Water solubility (mg/L)	5.445×10^{-2}	WSKOWWIN 2010
	Log K_{ow}	6.60	KOWWIN 2010
	Log K_{oc}	5.17	KOCWIN 2010
5290-62-0 Magneson II (C)	Melting point (°C)	194.88	MPBPWIN 2010
	Vapour pressure (Pa)	8.40×10^{-8}	MPBPWIN 2010
	Henry's Law constant ($Pa \cdot m^3/mol$)	5.97×10^{-8}	HENRYWIN 2011
	Water solubility (mg/L)	0.6814	WSKOWWIN 2010
	Log K_{ow}	5.20	KOWWIN 2010
	Log K_{oc}	4.62	KOCWIN 2010
6535-42-8 Solvent Red 3 (C)	Melting point (°C)	168.17	MPBPWIN 2010
	Vapour pressure (Pa)	6.08×10^{-7}	MPBPWIN 2010
	Henry's Law constant ($Pa \cdot m^3/mol$)	1.19×10^{-6}	HENRYWIN 2011
	Water solubility (mg/L)	0.4876	WSKOWWIN 2010
	Log K_{ow}	5.38	KOWWIN 2010
	Log K_{oc}	4.55	KOCWIN 2010
2653-64-7 Solvent Red 4 (C)	Melting point (°C)	191.42	MPBPWIN 2010
	Vapour pressure (Pa)	3.56×10^{-8}	MPBPWIN 2010
	Henry's Law constant ($Pa \cdot m^3/mol$)	1.48×10^{-6}	HENRYWIN 2011
	Water solubility (mg/L)	0.002 689	WSKOWWIN 2010
	Log K_{ow}	6.68	KOWWIN 2010
	Log K_{oc}	5.22	KOCWIN 2010
6407-78-9 Solvent Yellow 18 (D)	Melting point (°C)	196.47	MPBPWIN 2010
	Vapour pressure (Pa)	4.23×10^{-7}	MPBPWIN 2010
	Henry's Law constant ($Pa \cdot m^3/mol$)	1.76×10^{-4}	HENRYWIN 2011

CAS RN, C.I. name (eco-subset)	Property	Value	Reference
	Water solubility (mg/L)	6.294×10^{-2}	WSKOWWIN 2010
	Log K_{ow}	5.65	KOWWIN 2010
	Log K_{oc}	4.46	KOCWIN 2010

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

Appendix C. Empirical and Modelled Data for Degradation of the Azo Solvent Dyes

Table C-1: Empirical data for degradation of the Azo Solvent Dyes

C.I. name	Medium	Fate process	Degradation value	Degradation endpoint/unit	Reference
Solvent Yellow 1	Wastewater	Biodegradation	89% (50 mg/L)	% degradation—13 days	Urushigawa and Yonezawa 1977
	Wastewater	Biodegradation	0% BOD	% biodegradation— 5 and 6 days	Heuelekian and Rand 1955
	Wastewater	Biodegradation	0% BOD	% biodegradaton—5 days	Niemi et al. 1987
	Water	Biodegradation by white rot basidiomycete *Phanerochaete*	25.8% (low-nitrogen culture) 4.7% (high-nitrogen culture)	% biodegradation—12 days	Spadaro et al. 1992
	Wastewater	Biodegradation	46% (HPLC) (100 ppm)	% biodegradation—24 h	Idaka and Ogawa 1978
	Wastewater	Biodegradation	59% (HPLC) (100 ppm)	% biodegradation—48 h	Idaka and Ogawa 1978
	Water	Biodegradation	89%	% biodegradation—13 days	HSDB 1983–
	Wastewater	Biodegradation	< 10%	% CO_2 evolution—28 days	ECHA ©2007–2013
Solvent Yellow 2	Water	Biodegradation	100%	% biodegradation—28 days	Fochtman 1981
	Water	Biodegradation	100%	% biodegradation— 7 days	HSDB 1983–
Azobenzene	Water	Biodegradation	50%	% biodegradation—1.4 days	Zoeteman et al. 1980
	Wastewater	Oxidation	225 mg/L (resistant to degradation)	Oxygen uptake—8 days	Malaney 1960
	Beaver dam water	Biodegradation	50%	% biodegradation—13.8 h	Weber and Wolfe 1987
	Beaver dam water	Biodegradation	50% (autoclaved)	% biodegradation—163.0 h	Weber and Wolfe 1987
	Beaver dam water	Biodegradation	50% (treated with 0.025 M formaldehyde)	% biodegradation—16.8 h	Weber and Wolfe 1987
	Beaver dam	Biodegradation	50% (treated	%	Weber and

C.I. name	Medium	Fate process	Degradation value	Degradation endpoint/unit	Reference
	water		with 0.09 M sodium azide)	biodegradation—24.4 h	Wolfe 1987
	Beaver dam water	Biodegradation	50% (treated with 0.09 M *m*-cresol)	% biodegradation—15.2 h	Weber and Wolfe 1987

Abbreviations: BOD, biological oxygen demand; CAS RN, Chemical Abstracts Service Registry Number; HPLC, high-performance liquid chromatography; OC, organic carbon; ppm, parts per million; UV, ultraviolet

Table C-2: Summary of modelled data for degradation of the 15 monoazo solvent dyes[a]

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Air			
Atmospheric oxidation	AOPWIN 2010[b]	$t_{½}$ = 0.053–6.921 days	≤ 2 to ≥ 2
Ozone reaction	AOPWIN 2010[b]	NA[c]	NA
Water			
Hydrolysis	HYDROWIN 2010[b]	Not in training set	NA
Primary biodegradation			
Biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 4: Expert Survey (qualitative results)	[3.1868–3.583][d] "moderate degradation to may degrade fast"	≤ 182
Ultimate biodegradation			
Biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 3: Expert Survey (qualitative results)	[2.1163–2.7816][d] "borderline slow biodegradation to may degrade fast"	Most substances ≥ 182
Biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 5: MITI linear probability	[0.0043–0.2474][e] "biodegrades slowly to may degrade fast"	Most substances ≥ 182
Biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 6: MITI non-linear probability	[0–0.6936][e] "biodegrades slowly to may degrade fast"	Most substances ≥ 182
Biodegradation (aerobic)	DS TOPKAT ©2005– 2009 Probability	N/A	
Biodegradation (aerobic)	CATALOGIC (2012) % BOD	% BOD = [0.56–12.2] "degrades slowly"	≥ 182

Abbreviations: BOD, biological oxygen demand; MITI, Ministry of International Trade & Industry, Japan; NA, not available; N/A, not applicable; $t_{½}$, half-life

[a] Substances used in this summary include the following C.I. names: Solventy Yellow 1, Solvent Yellow 2, Solvent Yellow 3, Azobenzene, Solvent Orange 3, Solvent Yellow 77, 4-Anilinoazobenzene, Solvent Yellow 18, Solvent Yellow 14, Solvent Red 1, Solvent Orange 2, Solvent Orange 7, Magneson II, Solvent Red 3, Solvent Red 4.

[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 C-3: Summary of modelled data for degradation of the seven disazo solvent dyes[a]

Fate process	Model and model basis	Model result and prediction	Extrapolated half-life (days)
Air			
Atmospheric oxidation	AOPWIN 2010[b]	$t_{½}$ = 0.053–1.156 days	≤ 2
Ozone reaction	AOPWIN 2010[b]	NA[c]	NA
Water			
Hydrolysis	HYDROWIN 2010[b]	NA	NA
Primary biodegradation			
Biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 4: Expert Survey (qualitative results)	[2.1359–3.2783][d] "biodegrades slowly to moderate degradation "	≥ 182
Ultimate biodegradation			
Biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 3: Expert Survey (qualitative results)	[−0.1879–1.8981][d] "biodegrades slowly"	≥ 182
Biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 5: MITI linear probability	[−1.0884 to −0.8839][e] "biodegrades slowly"	≥ 182
Biodegradation (aerobic)	BIOWIN 2010[b] Sub-model 6: MITI non-linear probability	[0][e] "biodegrades slowly"	≥ 182
Biodegradation (aerobic)	DS TOPKAT ©2005–2009 Probability	N/A	
Biodegradation (aerobic)	CATALOGIC (2012) % BOD	% BOD = [2.2–8.8] "biodegrades slowly"	≥ 182

Abbreviations: BOD, biological oxygen demand; MITI, Ministry of International Trade & Industry, Japan; NA, not available; N/A, not applicable; $t_{½}$, half-life

[a] Substances used in this summary include the following CAS RNs or C.I. names: 73528-78-6, 85392-21-8, 21519-06-2, Solvent Red 24, Solvent Red 23, Solvent Red 19, 73507-36-5.

[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.

Appendix D. Empirical Data for the Aquatic Toxicity of Azo Dyes and Analogues

Table D-1: Empirical data for the aquatic toxicity of Azo Solvent Dyes used in the ecological assessment

C.I. name, eco-subset	Test organism	Type of test	Endpoint	Value (mg/L)	Reference
Solvent Yellow 1 (A)	Fish *Oryzias latipes*	Acute 96 h	LC_{50}	0.23	CHRIP ©2008
		Acute 96 h	LC_{50}	0.35	CHRIP ©2008
		Acute 24 h	LC_{50}	1.7	Tonogai et al. 1982
		Acute 48 h	LC_{50}	0.7	Tonogai et al. 1982
	Protozoa *Tetrahymena pyriformis*	Acute 48 h	IGC_{50}	6.38	Schultz 1997
	Alga *Pseudokirchneriella subcapitata*	Acute 72 h	EC_{50} (growth rate)	2.9	CHRIP ©2008
		Acute 72 h	NOEC (growth rate)	0.14	CHRIP ©2008
		Acute 72 h	EC_{50}	1.2	CHRIP ©2008
		Acute 48 h	EC_{50} (acute immobilization)	0.46	CHRIP ©2008
	Invertebrate *Daphnia magna*	Chronic 21 days	EC_{50} (reproduction)	> 0.014	CHRIP ©2008
		Chronic 21 days	NOEC (reproduction)	0.0071	CHRIP ©2008
	Crustacea *Ceriodaphnia*	Acute 48 h	EC_{50}	0.07	BUA 2000
Solvent Yellow 2 (A)	Fish *Poecilia reticulata*	Chronic 365 days	Tumours	300 000 (30% mg)	Khudoley 1972
	Fish *Oryzias latipes*	Acute 24 h	LC_{50}	1.1	Tonogai et al. 1982
		Acute 48 h	LC_{50}	0.6	Tonogai et al. 1982
Azobenzene (A)	Fish *Oryzias latipes*	Acute 48 h	LC_{50}	0.5	Tonogai et al. 1982
		Acute 24 h	LC_{50}	1.00	Tonogai et al. 1982
	Invertebrate *Daphnia magna*	Chronic 24 days	EC_{50}	2.5	Kuhn et al. 1989
		Chronic 24 days	EC_{50}	5	Kuhn et al. 1989
		Acute 24 h	EC_{50}	0.13	Tosato et al. 1993
		Chronic 21 days	NOEC	0.023	Kuhn et al. 1988
		Chronic 21 days	NOEC (reproduction)	0.009	Kuhn et al. 1989
	Alga *Scenedesmus subspicatus*	Chronic 0–48 h	EC_{50} (cell growth)	1.7	Kuhn and Pattard 1990

C.I. name, eco-subset	Test organism	Type of test	Endpoint	Value (mg/L)	Reference
		Chronic 0–48 h	EC_{50} (growth rate)	2.5	Kuhn and Pattard 1990
Solvent Orange 3 (A)	Fish *Oryzias latipes*	Acute 24 h	LC_{50}	0.5	Tonogai et al. 1982
		Acute 48 h	LC_{50}	0.3	Tonogai et al. 1982
Solvent Red 1 (C)	Larval fish *Pimephales promelas*	Chronic 20 days	LC_{50}	0.0167	Parrott et al. 2014
	Invertebrate *Hyalella azteca*	Acute 1 week	LC_{50}	0.277	Bartlett 2014
	Invertebrate *Hyalella azteca*	Chronic 4 weeks	LC_{50}	0.0265	
Solvent Red 24 (E)	Fish *Oryzias latipes*	Acute 48 h	LC_{50}	> 100	MITI 1992

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index; EC_{50}, the concentration of a substance at which there is a sublethal effect observed on 50% of the test organisms within the test duration; IGC_{50}, inhibited the growth of cells by 50%; LC_{50}, the concentration of a substance at which there is a lethal effect observed on 50% of the test organisms within the test duration; NOEC, no-observed-effect concentration

Table D-2: Empirical data for the aquatic toxicity of analogues used in the ecological assessment

CAS RN, C.I. name, eco-subset	Test organism	Type of test	Endpoint	Value (mg/L)	Reference
85-84-7 Solvent Yellow 5 (C)	Fish *Oryzias latipes*	Acute 24 h	TL_m	0.9	Tonogai et al. 1982
		Acute 48 h	TL_m	0.5	Tonogai et al. 1982
131-79-3 Solvent Yellow 6 (C)	Fish *Oryzias latipes*	Acute 24 h	TL_m	0.6	Tonogai et al. 1982
		Acute 48 h	TL_m	0.4	Tonogai et al. 1982
1689-82-3 Solvent Yellow 7 (A)	Juvenile fish *Pimephales promelas*	Acute 96 h	LC_{50}	1.64	Broderius et al. 1995
	Fish *Pimephales promelas*	Acute 72 h	LC_{50}	1.10 (1.07–1.13)	Holcombe et al. 1984
		Acute 96 h	LC_{50}	1.09 (1.05–1.13)	Holcombe et al. 1984
		Acute 96 h	Hyperactivity, fish darting, rolling	1.38	Holcombe et al. 1984
		Acute 96 h	Some loss of equilibrium	0.93	Holcombe et al. 1984
	Fish *Poecilia reticulata*	Acute 96 h	LC_{50}	1.14	Raevsky and Dearden 2004
	Fish *Pimephales promelas*	Acute 96 h	LC_{50}	1.16	Schultz 1997
	Fish *Pimephales promelas*	Acute 96 h	LC_{50}	1.17	Russom et al. 1997

CAS RN, C.I. name, eco-subset	Test organism	Type of test	Endpoint	Value (mg/L)	Reference
	Freshwater ciliate	Acute 40 h	IGC_{50}	4.34	Schultz 1997
2610-11-9 Direct Red 81 (H)	Fish *Pimephales promelas*	Acute 96 h	TL_{50}	> 180	Little and Lamb 1972

Abbreviations: CAS RN, Chemical Abstracts Service Registry Number; C.I., Colour Index; IGC_{50}, inhibited the growth of cells by 50%; LC_{50}, the concentration of a substance at which there is a lethal effect observed on 50% of the test organisms within the test duration; TL_{50}or TL_m, median tolerable limit (lethal concentration for 50% of fish

Appendix E. Critical Body Burden Approach for the Azo Solvent Dyes

In terms of aquatic toxicity, the critical body burden (CBB) concept shows that an aquatic organism that takes up a chemical from water may accumulate this chemical until a certain critical body burden has been attained, causing the mortality of the organism. McCarty (1986, 1987a, b, 1990), McCarty and Mackay (1993), McCarty et al. (1985, 1991) and Van Hoogen and Opperhuizen (1988) have shown that internal concentrations of halogenated organic chemicals in fish causing death are fairly constant: about 2–8 mmol/kg.

Sijm and Hermens (2000) indicated that McCarty (1987a,b) and McCarty et al. (1991) have mathematically explained this as follows. The fairly constant internal effect concentration or lethal body burden (LBB) is the result of the bioconcentration factor (BCF), which increases with K_{ow}, and the external effect concentration (LC_{50}), which decreases with K_{ow}:

$$LBB = LC_{50} \times BCF$$

Therefore:

$$\log LBB \approx \log (LC_{50}) + \log (BCF) \approx (-\log K_{ow} + b_1) + (\log K_{ow} + b_2) \approx b_1 + b_2 \approx \text{constant}$$

where b_1 and b_2 are constants.

Having analyzed the literature data, Sijm and Hermens (2000) emphasized that for narcotic (e.g., polychlorinated benzenes and biphenyls) and polar-narcotic compounds (e.g., chlorinated phenols and anilines), sufficient information is available to study this assumption. The authors came to the conclusion that for different organisms, the lethal body burdens for polar narcotics vary by approximately two orders of magnitude, and thus again show a significant reduction in the variation of the ecotoxicological effect concentrations, compared with the more than five orders of magnitude differences that are found in external effect concentrations for this type of mechanism of action.

While applying a CBB approach for the Azo Solvent Dyes, the following assumptions have been made: 1) for the CBB calculations for Solvent Yellow 1 and Solvent Red 24, the lipid concentration of the fish in the BCF tests is 5%; 2) Disperse Red 167, Disperse Yellow 163 and Disperse Orange 73 are suitable Azo Solvent Dye analogues, as there are limited BCF studies on solvent dyes; 3) the dyes are not reactive or specifically acting reactive chemicals, i.e., they provoke toxicity only through non-specific mechanisms (i.e., narcotic mode of action); 4) there are no dye–dispersant (or dye–solvent) interactions; 5) the purity of dye is very high; 6) for lethal effects, once the aquatic organism has reached the lethal body burden, it dies; and 8) the average acute CBB threshold is 5 mmol/kg.

Critical Body Burden (CBB) and External Effect Concentration (EEC) Calculations

Since CBB = LC_{50} × BCF (see above), the expected external acute effect concentration (LC_{50}) can be back-calculated as:

$$LC_{50}\,(mmol/L) = \frac{CBB\,(mmol/kg)}{BCF\,(L/kg)}$$

For Solvent Yellow 1, definitive experimental whole-body BCF data are available (see Table 5-2): 37.3 L/kg at 6 µg/L and < 31.6 L/kg at 0.6 µg/L. Therefore, the average whole-body BCF is 34.45 L/kg.

However, the BCF in fish is usually normalized for the 5% lipid content of the organism:

$$BCF_L = \frac{BCF_{wb\ ww}}{L_f} \times 5\%$$

where BCF_L is the lipid-normalized bioconcentration factor, $BCF_{wb\ ww}$ is the whole-body BCF (wet weight basis), L_f is the lipid content (fraction) in the organism and 5% is the generally accepted lipid level for lipid-normalized BCF.

If so, applying a lipid level in fish of 5%, the lipid-normalized BCF is:

$$BCF_L = \frac{34.45\,(L/kg)}{0.05} \times 0.05 \approx 34.45\ \text{L/kg}$$

Therefore, using the average acute CBB threshold of 5 mmol/kg, the external effect concentration can be calculated as:

$$\text{Acute } LC_{50} = \frac{CBB\,(mmol/kg)}{BCF\,(L/kg)} = \frac{5\,(mmol/kg)}{34.45\,(L/kg)} = 0.145\ \text{mmol/L}$$

Considering that the molecular weight of Solvent Yellow 1 is ~197.24 g/mol (or 197.24 mg/mmol), the external acute effect concentration, expressed in mg/L, is:

0.145 mmol/L × 197.24 mg/mmol = 28.6 mg/L

Similarly, external acute effect concentrations can be calculated for other solvent dyes as well as disperse dye analogues. The results are presented in Table 6-3.

Appendix F. Ecological Exposure Calculations for Azo Solvent Dyes

The predicted environmental concentrations (PECs) were estimated in water, sediment and soil for the two formulation scenarios identified. Sites were identified for each scenario according to the responses to an information request from Environment Canada and Health Canada (2013 emails from importing companies to Environment Canada; unreferenced). These two scenarios include:

1) formulation of adhesives;
2) formulation of lawn and garden care products.

Estimates for Aquatic PECs

The aquatic PEC was estimated based on a number of parameters: the annual quantity of the Azo Solvent Dyes used, the number of annual operation days involved with these solvent dyes, emission factor to wastewater, removal by wastewater treatment systems, wastewater flow and receiving water dilution. The approach used in the calculations was to determine the concentration of the Azo Solvent Dyes near the wastewater treatment system's effluent discharge point based on the wastewater flow and receiving water dilution.

The annual use quantity of the Azo Solvent Dyes at a facility was reported to be between 100 and 1000 kg for the formulation of adhesives and between 10 and 100 kg for the formulation of lawn and garden products (2013 email from company to Environment Canada; unreferenced). The actual annual use quantities of the Azo Solvent Dyes were used to determine the respective aquatic PECs.

The number of annual operation days relevant to these solvent dyes was unknown for the formulation of adhesives or lawn and garden products. The European Commission Joint Research Centre (ECJRC 2003) provided estimated numbers of annual formulation days based on annual use quantities. The estimated numbers of days for an annual use quantity between 10 and 1000 kg for the industry categories applicable to the formulation scenarios are given below:

- 1 day/year at an annual use quantity between 10 and 500 kg;
- 2 days/year at an annual use quantity between 500 and 1000 kg.

These estimates were used to convert an annual use quantity to a daily use quantity for the formulation of adhesives or lawn and garden products. For a hypothetical annual use quantity of 100 kg, the daily use quantity of the Azo Solvent Dyes was estimated by dividing the annual use quantity by an applicable number of annual operation days:

Daily use quantity of Azo Solvent Dyes (for formulation of adhesives or lawn and garden products)

= Annual use quantity / Number of annual operation days

= 100 kg/year / 1 day/year
= 100 kg/day

The emission factor to wastewater for the formulation of adhesives or lawn and garden products was unknown. It was estimated using the guidance from the ECJRC (2003). The estimated emission factor was 2% when a substance's annual quantity was below 1 000 000 kg. This 2% estimate was used for the formulation of adhesives or lawn and garden products.

Emission factor to wastewater (for formulation of adhesives or lawn and garden products) = 2%

The daily release quantity of the Azo Solvent Dyes to wastewater from a formulation site was estimated by multiplying the daily use quantity by the emission factor to wastewater.

It was not known whether on-site industrial wastewater treatment was used at sites for the formulation of adhesives and lawn and garden products. In the absence of this information, it was conservatively assumed that there was no on-site wastewater treatment.

The daily release quantity of the Azo Solvent Dyes to the sewer system from the formulation of adhesives or lawn and garden products was simply equal to the daily release quantity to wastewater, because no on-site wastewater treatment was assumed.

Daily release quantity of Azo Solvent Dyes to sewer system (for formulation of adhesives or lawn and garden products)
= Daily release quantity of Azo Solvent Dyes to wastewater (for formulation of adhesives or lawn and garden products)

The concentration of the Azo Solvent Dyes in wastewater influent depends upon the flow of a local wastewater treatment system.

The removal efficiencies of the local wastewater treatment systems at the sites were estimated by models. The treatment systems used at these sites were all secondary, and their removal efficiencies were estimated by ASTreat (2006). The five solvent dyes identified in commerce from a survey issued pursuant to section 71 of CEPA 1999 (Canada 2006; Environment Canada 2006) and the DSL Inventory Update survey (Canada 2009; Environment Canada 2009) were non-volatile and were assumed not to biodegrade through wastewater treatment, due to a lack of biodegradation data. The removal efficiencies estimated were therefore a result of sludge removal only. The estimate, given below, was specific to the Azo Solvent Dyes used at each site:

Removal = 88.6% for formulation of adhesives or lawn and garden products

The concentration of the Azo Solvent Dyes in wastewater effluent was estimated from the concentration of solvent dyes in wastewater influent and an applicable off-site local wastewater treatment removal efficiency.

The aquatic PEC was estimated by dividing the effluent concentration by an appropriate dilution factor of the receiving water. Since the aquatic PEC is determined near the discharge point, the receiving water dilution selected should be applicable to this requirement. The full dilution potential of a river is considered appropriate if it is between 1 and 10. Otherwise, the 10-fold dilution is used for both large rivers and still waters.

The aquatic PEC results for the two formulation scenarios were 0.40 µg/L and 0.46 µg/L.

Estimates for Sediment PECs

An equilibrium sediment–water partition approach described by the European Chemicals Agency (ECHA 2010) was used to estimate the concentration of the solvent dyes in sediment. This approach assumes that the concentration in bottom sediment is in equilibrium with the concentration in the overlying water. At equilibrium, the PEC in bottom sediment can linearly correlate with the concentration in the aqueous phase of the overlying water as follows:

$$\text{Sediment PEC} = K_{sw}C_w$$

where:

K_{sw}: sediment–water partition coefficient (L/kg)
C_w: chemical concentration in aqueous phase (mg/L)

The sediment–water partition coefficient (K_{sw}, L/kg) can be estimated from the organic carbon (OC) fraction of sediment (F_{oc}, kg OC/kg), the sorptive capacity of sediment's OC (A_{oc}, L/kg OC) and a substance's octanol–water partition coefficient (K_{ow}, unitless) (Gobas 2010):

$$K_{sw} = F_{oc}A_{oc}K_{ow}$$

The sediment PEC can then be calculated from the equation:

$$\text{Sediment PEC} = F_{oc}A_{oc}K_{ow}C_w$$

The concentration in the aqueous phase (C_w, mg/L) can be estimated from the aquatic PEC (mg/L). There are three distinct phases in the water column: aqueous, particulate suspended sediment and dissolved suspended sediment (Gobas 2007). Accordingly, the total concentration in the water column or the aquatic PEC (mg/L) can be expressed as a sum of the concentrations in the aqueous phase (C_w, mg/L), particulate suspended sediment (C_{ps}, mg/L) and dissolved suspended sediment (C_{ds}, mg/L):

$$\text{Aquatic PEC} = C_w + C_{ps} + C_{ds}$$

When the OC phase in particulate or dissolved suspended sediment is the phase of sorption for a substance, the above equation can be converted to an expression for estimating the ratio of the aquatic PEC (mg/L) to the concentration in the aqueous phase (C_w, mg/L) (Gobas 2007):

$$\text{Aquatic PEC}/C_w = 1 + (X_{ps}F_{poc}A_{poc} + X_{ds}F_{doc}A_{doc})K_{ow}$$

where:

X_{ps}: content of particulate suspended sediment in water column (kg/L)
F_{poc}: OC fraction of particulate suspended sediment (kg OC/kg)
A_{poc}: sorptive capacity of particulate OC relative to octanol (L/kg OC)
X_{ds}: content of dissolved suspended sediment in water column (kg/L)
F_{doc}: OC fraction of dissolved suspended sediment (kg OC/kg)
A_{doc}: sorptive capacity of dissolved OC relative to octanol (L/kg OC)
K_{ow}: octanol–water partition coefficient (unitless)

In Canada, the middle level for the content of particulate suspended sediment in the water column (X_{ps}) was 47 mg/L (i.e., 50th percentile; Environment Canada 2013). This value was used in the derivation of the sediment PECs.

$$X_{ps} = 47 \text{ mg/L} = 4.7 \times 10^{-5} \text{ kg/L}$$

According to Gobas (2010), the OC fraction of particulate suspended sediment varied from 0.1 to 0.2 kg/kg sediment. The lower end of this range was used in order to derive conservative sediment PECs.

$$F_{poc} = 0.1 \text{ kg OC/kg}$$

Karickhoff (1981) proposed a value of 0.41 L/kg OC for the sorptive capacity of sediment's OC based on a set of 17 sediment and soil samples and various hydrophobic non-polar organic compounds. This value was used for the sorptive capacity of particulate OC (A_{poc}).

$$A_{poc} = 0.41 \text{ L/kg OC}$$

In Canada, the dissolved OC content in the water column averaged 2.7 mg OC/L (Environment Canada 2013). This value was used in the derivation of the sediment PECs. Note that this OC content equals the product of the content of dissolved suspended sediment, X_{ds} (mg/L), and the OC fraction of dissolved suspended sediment, F_{doc} (kg OC/kg).

$$X_{ds}F_{doc} = 2.7 \text{ mg OC/L} = 2.7 \times 10^{-6} \text{ kg OC/L}$$

Gobas (2007) provided an estimate of 0.08 L/kg OC for the sorptive capacity of dissolved OC. This estimate was used.

A_{doc} = 0.08 L/kg OC

The octanol–water partition coefficient (K_{ow}) exhibits a significant influence on the sediment PEC. According to the following two equations, described previously:

Sediment PEC = $F_{oc}A_{oc}K_{ow}C_w$

Aquatic PEC/C_w = 1 + ($X_{ps}F_{poc}A_{poc}$ + $X_{ds}F_{doc}A_{doc}$)K_{ow}

the dependence of the sediment PEC on K_{ow} is given as:

Sediment PEC = Aquatic PEC × $F_{oc}A_{oc}$/(1/K_{ow} + $X_{ps}F_{poc}A_{poc}$ + $X_{ds}F_{doc}A_{doc}$)

This dependence reveals that the sediment PEC approaches zero for water-soluble substances with low K_{ow} and approaches a maximum constant concentration for highly hydrophobic substances with high K_{ow}. In other words, the sediment PEC increases with K_{ow}. The log K_{ow} values for the five Azo Solvent Dyes found in commerce were in the range of 3.6–6.1. The higher-end value of this range was used to derive conservative sediment PECs.

log K_{ow} = 6.1, or K_{ow} = 1 258 925

The ratio of the aquatic PEC to the concentration in the aqueous phase (C_w) was calculated as:

Aquatic PEC/C_w = 1 + ($X_{ps}F_{poc}A_{poc}$ + $X_{ds}F_{doc}A_{doc}$)K_{ow}
= 1 + [(4.7 × 10^{-5} kg/L × 0.1 kg OC/kg × 0.41 L/kg OC) + (2.7 × 10^{-6} kg OC/L × 0.08 L/kg OC) × 1 258 925]
= 1 + (2.14 × 10^{-6}) × 1 258 925
= 1 + 2.69 = 3.69

As an example, if the aquatic PEC at a site is estimated as 10.8 µg/L, the concentration in the aqueous phase (C_w) at this site is then calculated from the ratio of the aquatic PEC to C_w:

C_w = Aquatic PEC/3.69 = 10.8 µg/L / 3.69 = 2.93 µg/L

Gobas (2010) suggested a default value of 0.01–0.03 kg OC/kg for the OC fraction of bottom sediment in rivers. The higher end of this range was selected as a standard for the sediment PECs derived.

F_{oc} = 0.03 kg OC/kg

As for particulate suspended sediment, the sorptive capacity of bottom sediment's OC was taken as 0.41 L/kg OC based on the work from Karickhoff (1981).

A_{oc} = 0.41 L/kg OC

The sediment PEC at the site can then be estimated from the above values as:

Sediment PEC = $F_{oc}A_{oc}K_{ow}C_w$
= 0.03 kg OC/kg × 0.41 L/kg OC × 1 258 925 × 2.93 µg/L
= 15 485 L/kg × 2.93 µg/L
= 45 370 µg/kg
= 45.4 mg/kg

The sediment PECs were estimated according to the above method to be 1.66 mg/kg and 1.94 mg/kg.

Estimates for Soil PECs

An approach described by the European Chemicals Agency (ECHA 2010) was used to estimate PECs in soil resulting from the land application of sewage biosolids. This approach employed the quantity of biosolids accumulated within the top 20 cm layer (ploughing depth) of soil over 10 consecutive years as the basis for soil PECs. One underlying assumption of the approach was that substances were subject to no loss due to degradation, volatilization, leaching or soil runoff upon their entry into soil via biosolids land application. This assumption therefore yielded conservative soil PECs.

When the above conservative approach was applied to the Azo Solvent Dyes, the concentration of the Azo Solvent Dyes in biosolids was first estimated at a site. The data required for this estimate included the daily quantity of the Azo Solvent Dyes released to the sewer system at a site, the sludge removal efficiency of the related local wastewater treatment system, the per capita sludge production rate and the population served by the wastewater treatment system.

The daily quantity of the Azo Solvent Dyes released to the sewer system was estimated in the aquatic PEC calculations.

The removal efficiencies of the local wastewater treatment systems at sites were estimated by models. The treatment systems used at sites were all secondary, and their removal efficiencies were estimated by ASTreat (2006). The five Azo Solvent Dyes identified in commerce from a survey issued pursuant to section 71 of ECPA 1999 (Canada 2006; Environment Canada 2006) and the DSL Inventory Update survey (Canada 2009; Environment Canada 2009) were non-volatile and were assumed not to biodegrade through wastewater treatment, due to a lack of biodegradation data. The removal efficiencies estimated were therefore a result of sludge sorption only. These estimates ranged from 26.2% to 88.6%, and the higher-end value of 88.6% was used in order to derive conservative soil PECs.

Off-site local wastewater treatment removal = 88.6%

The daily quantity of the Azo Solvent Dyes sorbed to sludge was estimated by multiplying the daily release quantity by the removal:

= Daily release quantity of Azo Solvent Dyes to sewer system (kg/day) × Off-site local wastewater treatment removal (kg/day)

The per capita sludge production rate depends upon the type of wastewater treatment. This rate was reported to be 0.080 kg/day per person for primary sludge and 0.115 kg/day per person for secondary sludge (Droste 1997). In other words, the per capita sludge production rate was 0.195 kg/day per person from secondary systems (primary sludge rate at 0.080 kg/day per person + secondary sludge rate at 0.115 kg/day per person). The higher rate from secondary systems was mainly attributed to the biomass production during biological treatment.

Per capita sludge production rate from secondary systems = 0.195 kg/day per person

As an approximation, the daily quantity of biosolids produced from a wastewater treatment system was assumed to equal the daily quantity of sludge produced. This daily quantity was calculated by multiplying the sludge production rate by the population served by the wastewater treatment system:

Daily quantity of biosolids produced from a secondary system
= Per capita sludge production rate from a secondary system (kg/day per person) × Population served by the system (number of persons)

The concentration of the Azo Solvent Dyes in biosolids was obtained by dividing the daily quantity of the Azo Solvent Dyes sorbed to sludge by the daily quantity of biosolids produced from a wastewater treatment system.

Concentration of Azo Solvent Dyes in biosolids
= Daily quantity of Azo Solvent Dyes sorbed to sludge (kg/day) ÷ Daily quantity of biosolids produced from a secondary system (kg/day)
= g/kg

The annual quantity of the Azo Solvent Dyes entering soil via biosolids land application is a function of not only the concentration of the solvent dyes in biosolids, but also the biosolids application rate. In Canada, the use of biosolids is regulated by the provinces and territories. The rate at which biosolids are land applied can therefore vary between different provinces and territories, and are summarized below for four provinces.

Land application rates for sewage biosolids in Canada

Province	Application rate (t/ha)	Application period (years)	Annual application rate (t/ha per year)	Reference
Ontario	8	5	1.6	MOE and OMAFRA 1996
Quebec	22	5	4.4	MENV 2004
British Columbia	17	5	3.4	McDougall and Van Ham 2002
Alberta	25	3	8.3	Alberta Environment 2009

The annual quantity of the Azo Solvent Dyes entering soil via biosolids land application was calculated by multiplying the concentration of the Azo Solvent Dyes in biosolids by the maximum annual application rate found from the four provinces shown above.

Annual quantity of Azo Solvent Dyes entering soil

= Concentration of Azo Solvent Dyes in biosolids (g/kg) × Maximum biosolids annual application rate (8.3 t/ha per year)
= g/m^2 per year

According to the approach described by the European Chemicals Agency (ECHA 2010), a period of 10 consecutive years was used to determine the quantity of the Azo Solvent Dyes accumulated over this period.

Quantity of Azo Solvent Dyes accumulated in soil over 10 years

= Annual quantity of Azo Solvent Dyes entering soil (g/m^2 per year) × 10 years
= g/m^2

To derive the concentration of the Azo Solvent Dyes in soil, the quantity of soil within the top 20 cm or 0.20 m layer, as per the European Chemicals Agency (ECHA 2010), was estimated from a dry soil density of 1200 kg/m^3 (Williams 1999):

Quantity of soil = Soil depth × Soil density
= 0.20 m × 1200 kg/m^3
= 240 kg/m^2

The soil PEC at a site was then estimated by dividing the quantity of the Azo Solvent Dyes accumulated in soil over 10 years by the quantity of soil:

Soil PEC for Azo Solvent Dyes
= Quantity of Azo Solvent Dyes accumulated in soil over 10 years (g/m^2) / Quantity of soil (240 kg/m^2)

The soil PECs for all the sites were estimated according to the above method to be 0.31 mg/kg and 0.59 mg/kg.

Appendix G. Estimated Exposures from Use of Products

Table G-1: Summary of upper-bounding estimates of dermal exposure to Azo Solvent Dyes via uses of consumer or cosmetic products in Canada[a]

Exposure scenario[b]	Azo Solvent Dye	Concentration (% w/w)	Upper-bounding exposure estimate	
			Per event (mg/kg-bw)	Daily (mg/kg-bw per day)
Hair conditioner	Solvent Red 1	≤ 0.1	N/A	5.3×10^{-4}
Spray perfume	Solvent Red 3	≤ 0.1	N/A	2.1×10^{-3}
Essential oil: massage[c]	Solvent Red 3	≤ 0.1	N/A	1.9×10^{-3}
Leave-in hair conditioner	Solvent Yellow 18	≤ 0.1	N/A	5.3×10^{-3}
Textiles: personal apparel worn by adults	Solvent Yellow 77	1	N/A	5.5×10^{-3}
Textiles: baby sleeper	Solvent Yellow 77	1	N/A	8.7×10^{-3}
Leather furniture	Solvent Yellow 77	2	2.6×10^{-2}	N/A
Shoe polish[c]	Solvent Orange 3	≤ 1.5	0.021	0.0015
Writing ink (toddler)	Sudan I	1–5	2.1×10^{-3}	N/A

Abbreviations: kg-bw, kilograms of body weight; N/A, not applicable; w/w, weight per weight
[a] Exposure estimates are calculated using ConsExpov4.1 (ConsExpo 2006) unless otherwise specified. Refer to Appendix G for exposure factors.
[b] Exposure scenarios consider adults 20–59 years of age unless otherwise specified.
[c] The estimated daily exposure derives from one year's use amortized over 365 days.

Table G-2: Summary of upper-bounding estimates of oral exposure to Azo Solvent Dyes via use of consumer products[a]

Exposure scenario[b]	Azo Solvent Dye	Concentration (% w/w)	Upper-bounding exposure estimate	
			Per event (mg/kg-bw)	Daily (mg/kg-bw per day)
Mouthing of textile objects by an infant[c]	Solvent Yellow 77	1	N/A	2.7×10^{-4}
Incidental ingestion of paper by a toddler[c]	Solvent Yellow 77	5	3.2	N/A
Incidental ingestion of writing ink (via hand-to-mouth activity; toddler)[d]	Sudan I	1–5	8.1×10^{-3}	N/A

Abbreviations: kg-bw, kilograms of body weight; N/A, not applicable; w/w, weight per weight
[a] Exposure estimates are calculated using ConsExpov4.1 (ConsExpo 2006)
[b] Exposure scenarios consider adults 20–59 years of age unless otherwise specified.
[c] Refer to Appendix G.
[d] Refer to Appendix G.

Table G-3 Exposure factors for estimating dermal exposures via use of cosmetics products

Product scenario	Exposure factors[a]
Depilatory cream	Exposure frequency: 0.0466/day

Product scenario	Exposure factors[a]
	Product amount: 5.5 g/application Overall retention factor: 0.1 (US EPA 2011) Concentration: ≤ 0.3% (Solvent Red 3)
Essential oil: massage	Exposure frequency: 0.0658/day Product amount: 8 g/application Overall retention factor: 1 (Cadby et al. 2002; Wormuth et al. 2005; RIVM 2006a; SCCP 2006a; NICNAS 2009; SDA 2010a, b) Concentration: ≤ 0.1% (Solvent Red 3)
Hair conditioner	Exposure frequency: 1.1/day (Loretz et al. 2008) Product amount: 13.1 g/application (Loretz et al. 2008) Overall retention factor: 0.01 (Wormuth et al. 2005; SCCP 2006a; SDA 2010b) Concentration: ≤ 0.1% (Solvent Red 1, Solvent Yellow 18)
Hair straightener	*Assumption similar to a Hair Perm scenario.* Exposure frequency: 0.5/month (Wu et al. 2010) Product amount: 80 g/application (RIVM 2006a) Overall retention factor: 0.1 (professional judgement) Concentration: ≤ 0.1% (Solvent Red 1)
Leave-in hair conditioner	Exposure frequency: 1.1/day (Loretz et al. 2008) Product amount: 13.1 g/application (Loretz et al. 2008) Overall retention factor: 0.1 (professional judgement) Concentration: ≤ 0.1% (Solvent Yellow 18)
Manicure preparation gel	*Assumption similar to a Nail Polish scenario.* Exposure frequency: 151/year Product amount: 0.05 g/application Concentration: ≤ 0.1% (Solvent Red 1)
Soap, liquid form: showering	Exposure frequency: 0.901/day Product amount: 8.7 g/application Overall retention factor: 0.0033 (RIVM 2006a; SDA 2010a) Concentration: ≤ 0.1% (Solvent Red 3)
Soap, solid form: showering	Exposure frequency: 0.901/day Product amount: 7 g/application Overall retention factor: 0.0033 (Cadby et al. 2002; RIVM 2006a; SDA 2010a) Concentration: ≤ 0.3% (Solvent Red 1), ≤ 0.1% (Solvent Yellow 18)
Spray perfume	Exposure frequency: 1.7/day (Loretz et al. 2006) Product amount: 0.33 g/application (Loretz et al. 2006) Overall retention factor: 1 (Wormuth et al. 2005; SDA 2010a,b) Concentration: ≤ 0.1% (Solvent Red 3)

[a] All assumptions were ConsExpo default assumptions (RIVM 2006a) unless otherwise noted. In addition, the following assumptions were applied to all scenarios unless otherwise noted:

- Exposure scenarios are for an adult
- Adult body weight (20–59 years): 70.9 kg (Health Canada 1998)
- Based on notifications submitted under the *Cosmetic Regulations* to Health Canada (2011 and 2013 emails from the Consumer Product Safety Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada; unreferenced).
- Dermal absorption of 26% was applied for Solvent Red 1, Solvent Red 3 and Solvent Yellow 18 based on an *in vitro* study by Collier et al. (1993) for Sudan I.

Table G-4 Exposure factors for estimating inhalation exposures via use of cosmetics products

Product	Routes	Exposure factors
Spray perfume	Inhalation and oral	*ConsExpo v4.1: "Exposure, spray model"* Exposure frequency: 1.7/day (Loretz et al. 2006)

Product	Routes	Exposure factors
	non-respirable route	Concentration: ≤ 0.1% (Solvent Red 3) Inhalation rate: 16.2 m^3/day Spray duration: 0.08 min Exposure duration: 5 min Room volume: 10 m^3 Room height: 2.5 m Ventilation rate: 2/h Cloud volume: 0.0625 m^3 Mean mass generation rate: 0.0688 g/s (to give product amount of 0.33 g/application) Airborne fraction: 0.2 g/g Weight fraction non-volatile: 0.05 g/g Density non-volatile: 1.5 g/cm^3 Initial particle distribution median diameter (CV[b]): 2.7 µm (0.73) (RIVM 2010) Inhalation cut-off diameter: 15 µm

[a] All assumptions were ConsExpo default assumptions (RIVM 2006a) unless otherwise noted. In addition, the following assumptions were applied to all scenarios unless otherwise noted:

- Exposure scenarios are for an adult (20–59 years) with a body weight of 70.9 kg (Health Canada 1998)
- Based on notifications submitted under the *Cosmetic Regulations* to Health Canada (2011 and 2013 emails from the Consumer Product Safety Directorate, Health Canada, to the Existing Substances Risk Assessment Bureau, Health Canada; unreferenced).

[b] CV: coefficient of variation

Dermal and Oral Exposure from Textiles

Direct and prolonged skin contact with clothing may lead to dermal exposure to azo dyes. As a conservative approach, an upper-bounding exposure estimate to Solvent Yellow 77 based on full body coverage from wearing clothing is presented. It is assumed to account for exposures from multiple pieces of apparel that cover the entire surface area of the body. As Solvent Yellow 77 is relatively water soluble, the effect of laundering is expected to significantly reduce any dye that is not fixed to the textile fibre, thereby reducing the exposure after the initial washes. This effect was not factored into the estimated exposures; this adds to the conservatism of the estimates.

Estimated Daily Exposure via Dermal Route from Textile Apparel

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

Oral Exposure from Mouthing of Textile Objects by Infants

Oral exposure to Solvent Yellow 77 is estimated based on a scenario assuming that the infant is mouthing a textile object (e.g., blanket, textile toy) that may release Solvent Yellow 77. Conservatism is built in exposure factors described below.

Estimated Daily Exposure via Oral Route from Mouthing Textile Object

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

Exposure Factors

SA: Total surface area (dermal) = 18 200 cm^2 (adult), 3020 cm^2 (infant) (Health Canada1995)
Total surface area (oral: textile object mouthed) = 20 cm^2 (Zeilmaker et al. 1999)

AW: Area weight of textile = 20 mg/cm^2 (US EPA 2012a)
The area weight of textiles can vary greatly depending on the type of material. An area weight of 20 mg/cm^2 for cotton textiles is recommended by the US EPA in "Standard Operating Procedures for Residential Pesticide Exposure Assessment" (US EPA 2012a).

SCF: Skin contact factor = 1
Based on a conservative estimate that the 100% of the full body coverage of clothing being in direct contact with the skin (i.e., SCF = 1).

C: Concentration in textile = 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 dermal exposure from textiles is partly dictated by the amount of dye that migrates from textile material onto human skin (ETAD 1983b). The "Textiles" Working Group (BfR 2007) uses a peak initial migration of 0.5% to estimate exposure to dyes from newly bought unwashed garments. The migration rate after 28 hours of simulated wash and wear cycles was observed to be less than one-tenth of the value measured for the first migration. The migration fraction of 0.0005 which is one-tenth of the peak initial migration (0.5%) is used to reflect exposure after the intial 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).

UF: Dermal uptake fraction = 0.216 (Feldmann and Maibach 1970)
21.6% based on an in vivo dermal absorption study on Solvent Yellow 2.

F: Exposure frequency = 1×/day

P: Probability that Solvent Yellow 77 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. Solvent Yellow 77 does not derive from EU22 amines; the prevalence of this 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 Solvent Yellow 77 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 Solvent Yellow 77 every day are low.

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

Estimated Daily Exposures to Solvent Yellow 77 from Textiles via the Dermal Route

- Baby Sleeper: 8.7×10^{-4} mg/kg-bw per day
- Personal Apparel: 5.5×10^{-4} mg/kg-bw per day

Estimated Daily Exposure to Solvent Yellow 77 via Oral Route for Infants

- 2.7×10^{-5} mg/kg-bw per da

Dermal Exposure to Solvent Yellow 77 from Leather Products

Direct skin contact with articles of leather can result in dermal exposure to dyes used in leather dyeing. A number of leather products were considered in this exposure scenario, including those listed in the Danish EPA Annex XV Report on the proposed restriction of chromium in leather as leather articles (Danish EPA 2012). 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 hands can occur when playing with the toy. The presented exposure estimates below are considered upper-bounding based on conservative assumptions as well as not taking into account of a final application of a polyurethane sealant coating which would further reduce the consumer's dermal exposure to the leather dye.

Estimated Dermal Exposure to Solvent Yellow 77 from Leather Products

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

Exposure Factors

SA: Surface area of skin contact (Health Canada 1995; Therapeutic Guidelines Ltd. 2008).

- Shoes: 1275 cm^2 (adult feet)
- Boots: 4185 cm^2 (adult legs and feet)
- Gloves: 455 cm^2 (adult hands and forearms)
- Jackets and coats: 8920 cm^2 (adult trunk and arms)
- Trousers: 5820 cm^2 (adult lower body)

- Furniture: 5005 cm^2 (adult back, glutes and back of thighs)
- Toys: 92.5 cm^2 (infant palms)

AW: Area weight of leather = 0.15 g/cm^2 (Danish EPA 2012)
Based on the typical weight of 1 cm^2 leather (of 1 mm thickness) being 0.15 g, as in the leather shoe exposure scenario in the Danish EPA Annex XV Report on the proposed restriction of chromium in leather.

SCF: Skin contact factor = $[(SA_{direct} \times 1) + (SA_{indirect} \times 0.1)]/ (SA_{total})$
Based on a conservative assumptions and a weighted approach where SCF is assumed to be 1 when the entire leather product is in direct contact with the skin and SCF is assumd to be 0.1 when the leather product is in indirect contact with the skin (e.g., shielding due to interior lining) (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 based on surface areas (SA) of direct and indirect contact.

- Shoes: 1
- Boots: 0.1
- Gloves: 0.1
- Jackets and coats: 0.19
- Trousers: 0.19
- Furniture: 0.1
- Toys: 1

C: Concentration in material = 0.02 (unitless weight fraction) (Øllgaard et al. 1998)

M: Migration rate = 0.1% (derived from 39% over 365 days ; Zeilmaker et al. 1999)
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 migration of dyes from sweat. In estimating exposure to dyes from leather articles, it is assumed 39% of the dye content may leach over 1 year and is available for dermal exposure, which would be equivalent to 0.1% leaching over one day.

UF: Dermal uptake fraction = 0.216 (Feldmann and Maibach 1970)
21.6% based on an in vivo dermal absorption study on Solvent Yellow 2.

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

Exposure Estimates from Leather Products for Solvent Yellow 77

- Shoes: 1.2×10^{-2} mg/kg-bw
- Boots: 4.1×10^{-3} mg/kg-bw
- Gloves: 4.4×10^{-4} mg/kg-bw
- Jackets and coats: 1.7×10^{-2} mg/kg-bw
- Trousers: 1.1×10^{-2} mg/kg-bw
- Furniture: 4.9×10^{-3} mg/kg-bw
- Toys: 8.5×10^{-3} mg/kg-bw

Dermal exposure estimate to Solvent Orange 3 via use of shoe polish

Exposure estimate to Solvent Orange 3 via the use of shoe polish cream considering the potential subsequent exposure as described in the RIVM Cleaning Products Fact Sheet (RIVM 2006b).

Exposure frequency:	26/year (RIVM 2006b)
Product amount	0.10 g/application (RIVM 2006b)
Adult body weight (20–59 years):	70.9 kg (Health Canada 1998)
Concentration:	1.5% w/w (based on two shoe polish products at concentrations of 0–1% and 1.5%; HPDB 2012*).*
Dermal absorption:	100% (default)

Estimated Per Event Dermal Exposure to Solvent Orange 3 = 0.021 mg/kg-bws

Incidental dermal and oral exposure estimates to Sudan I from ballpoint pen ink

This scenario covers both dermal and incidental oral exposure from hand-to-mouth activity by a toddler. The Art and Creative Materials Institute, Duke University, Durham, North Carolina, reported the assumption that a child will absorb 25 cm of ink line daily either through dermal or incidental oral exposure (2009 personal communication from Art and Creative Materials Institute to Existing Substances Risk Assessment Bureau, Health Canada; unreferenced). A default ink laydown rate of 100 µg/cm (90th percentile level ink laydown of writing instruments; 2009 personal communication from Art and Creative Materials Institute to Existing Substances Risk Assessment Bureau, Health Canada; unreferenced) was used. Typically, the weight fraction of colourants in inks ranges from 1% to 5% (Scott and Moore 2000). In an *in vitro* study by Collier et al. (1993), 26% of applied Sudan I (5 µg/cm^2 in acetone) was absorbed by human skin within 24 hours; this percentage includes Sudan I in the skin sample (after homogenization) and in the receptor fluid and is expected to overestimate the dermal absorption of Sudan I in this scenario, since the contact is likely to occur over a shorter period. Overall, the resulting exposure estimate is expected to be upper bounding.

Estimated Upper-bounding Per Event Exposure via Dermal Route

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

Daily ink line:	25 cm
Ink laydown rate:	100 µg/cm
Concentration (*Conc*):	1–5% w/w (Scott and Moore 2000)
Dermal absorption fraction (*Dermal Abs*):	26% (Collier et al. 1993)
Body weight (*BW*):	15.5 kg

Estimated Per Event Dermal Exposure = 2.1×10^{-3} mg/kg-bw

Estimated Per Event Oral Exposure = 8.1×10^{-3} mg/kg-bw

Appendix H. Benchmark Dose Calculations for Solvent Yellow 77 and Sudan I

Table H-1: Summary of the calculated lowest BMD_{10} and $BMDL_{10}$ results[a]

Chemicals	CAS RN	Species	Tumours	BMD_{10} (mg/kg-bw per day)	$BMDL_{10}$ (mg/kg-bw per day)	Reference
Solvent Yellow 77	2832-40-8	Male F344 rats	Liver neoplastic nodules	78.38	51.29	NTP 1982a
Sudan I	842-07-9	Male F344 rats	Liver neoplastic nodules	9.31	5.54	NTP 1982b

Abbreviations: BMD_{10}, benchmark dose for a 10% response; $BMDL_{10}$, lower confidence limit on the benchmark dose for a 10% response; CAS RN, Chemical Abstracts Service Registry Number

[a] BMD_{10} and the corresponding $BMDL_{10}$ values were derived using the US EPA Benchmark Dose Software (BMDS version 2.3.1) (US EPA 2013). A dichotomous restricted model type was chosen for the BMD and BMDL analysis of cancer endpoints. Nine models were used for analysis of each tumour data set. These models included Gamma, Logistic, LogLogistic, LogProbit, Multistage, Multistage cancer, Probit, Weibull and Quantal-linear. The bset-fit models were selected and presented based on the highest p-value of goodness of fit and the lowest AIC value (a measure of information loss from a dose-response model that can be used to compare a set of models). In general, the p-value should be > 0.1 and the absolute value of SRI (represents observed minus predicted response divded by standard errors) should be < 2.

Solvent Yellow 77: The lowest calculated $BMDL_{10}$ for Sudan Yellow 77 is 51.29 mg/kg-bw per day for liver tumours in male rats, one of three tumour sites for this dye. The corresponding BMD_{10} is 78.38 mg/kg-bw per day. This $BMDL_{10}$ is selected for subsequent risk characterization.

Sudan I: The lowest calculated $BMDL_{10}$ for Sudan I is 5.54 mg/kg-bw per day for liver tumours in male rats, one of the four tumour endpoints for this dye. The corresponding BMD_{10} is 9.31 mg/kg-bw per day. This $BMDL_{10}$ is selected for subsequent risk characterization.

Benchmark Dose Calculations for Solvent Yellow 77 (CAS RN 2832-40-8)

Table H-2: Incidences of malignant tumours in rats or mice exposed to Solvent Yellow 77 in feeding diets (NTP 1982a)

	Incidence of malignant tumours		
	Low dose	Intermediate dose	High dose
Male F344 rats			
Dietary concentration (mg/kg)	0	5000	10 000
Equivalent dose (mg/kg-bw per day)[a]	0	250	500
Liver neoplastic nodules	1/49	15/50	10/50[b]
Liver neoplastic nodules or hepatocellular carcinoma	2/49	15/50	11/50[b]
Female B6C3F1 mice			
Dietary concentration (mg/kg)	0	2500	5000
Equivalent dose (mg/kg-bw per day)[a]	0	325	650
Hepatocellular adenoma	0/50	6/50	12/50

[a] Dose conversion was based on Health Canada (1994) guidance.

[b] When all doses included, *P* values < 0.01 and absolute scaled residual of interest (SRI) > 2. Thus, the highest dose point is deleted for subsequent calculations.

Table H-3: BMD_{10} and $BMDL_{10}$ calculations for tumours induced by Solvent Yellow 77 in rats and mice[a]

Tumours	Model name	No. of groups	AIC	*P*-value	SRI	BMR	BMD_{10} (mg/kg-bw per day)	$BMDL_{10}$ (mg/kg-bw per day)
Male rat liver neoplastic nodules	Quantal-linear	2	74.85	NA	0	0.1	78.38	51.29
Male rat liver neoplastic nodules or hepatocellular carcinoma	Quantal-linear	2	81.80	NA	0	0.1	83.61	53.22
Female mouse hepatocellular adenoma	Quantal-linear	3	93.82	0.99	−0.114	0.1	255.67	177.46

Abbreviations: AIC, Akaike's information criterion; BMD_{10}, benchmark dose for a 10% response; $BMDL_{10}$, lower confidence limit on the benchmark dose for a 10% response; BMR, benchmark response; SRI, scaled residual of interest

[a] A dichotomous restricted model type was chosen for the BMD and BMDL analysis of cancer endpoints. Nine models were used for analysis of each tumour data set. These models included Gamma, Logistic, LogLogistic, LogProbit, Multistage, Multistage cancer, Probit, Weibull and Quantal-linear. The bset-fit models were selected and presented here based on the highest *p*-value of goodness of fit and the lowest AIC value (a measure of information loss from a dose-response model that can be used to compare a set of models). In general, the *p*-value should be > 0.1 and the absolute value of SRI (represents observed minus predicted response divded by standard errors) should be < 2.

Benchmark Dose Calculations for Sudan I (CAS RN 842-07-9)

Table H-4: Incidences of tumours in F344 rats exposed to Sudan I in feeding diets (NTP 1982b)

	Incidence of tumours		
Dietary concentration (mg/kg)	0	250	500
Males			
Equivalent dose (mg/kg-bw per day)[a]	0	12.5	25
Liver neoplastic nodules	5/50	10/50	30/50
Liver neoplastic nodules or hepatocellular carcinoma	6/50	10/50	31/50
Females			
Equivalent dose (mg/kg-bw per day)[a]	0	12.5	25
Liver neoplastic nodules	2/50	3/49	10/48
Liver neoplastic nodules	2/50	3/49	11/48

[a] Dose conversion was based on Health Canada (1994) guidance.

Table H-5: BMD_{10} and $BMDL_{10}$ calculations for tumours induced by Sudan I in rats[a]

Tumours	Model name	No. of groups	AIC	*P*-value	SRI	BMR	BMD_{10} (mg/kg-bw per day)	$BMDL_{10}$ (mg/kg-bw per day)
Male rat liver neoplastic nodules	Multistage	3	154.67	0.371	0.749	0.1	9.31	5.54
Male rat liver neoplastic nodules or hepatocellular carcinoma	Multistage cancer	3	157.15	0.912	−0.09	0.1	12.53	6.31
Female rat liver neoplastic nodules	Multistage cancer	3	92.49	0.969	0.008	0.1	20.44	11.94
Female rat liver neoplastic nodules or hepatocellular carcinoma	Multistage cancer	3	95.05	0.912	0.024	0.1	19.58	11.88

Abbreviations: AIC, Akaike's information criterion; BMD_{10}, benchmark dose for a 10% response; $BMDL_{10}$, lower confidence limit on the benchmark dose for a 10% response; BMR, benchmark response; SRI, scaled residual of interest

[a] A dichotomous restricted model type was chosen for the BMD and BMDL analysis of cancer endpoints. Nine models were used for analysis of each tumour data set. These models included Gamma, Logistic, LogLogistic, LogProbit, Multistage, Multistage cancer, Probit, Weibull and Quantal-linear. The bset-fit models were selected and presented here based on the highest *p*-value of goodness of fit and the lowest AIC value (a measure of information loss from a dose-response model that can be used to compare a set of models). In general, the *p*-value should be > 0.1 and the absolute value of SRI (represents observed minus predicted response divded by standard errors) should be < 2.

Appendix I. Azo Solvent Dyes with Effects of Concern

Some of the Azo Solvent 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 I-1. Substances with effects of concern based on potential carcinogenicity

Substance Names and CAS RN	Classification for carcinogenicity[b]	Evidence of carcinogenicity from animal studies and/or human epidemiology	Release of EU22 aromatic amine by azo bond cleavage	Read-across
Azobenzene 103-33-3	EU Category 1B carcinogen US EPA Group B2 carcinogen	X		
Solvent Yellow 1 *p*-aminoazobenzene 60-09-3	IARC 2B EU Category 1B carcinogen	X	N/A (EU22)	
Solvent Yellow 2 60-11-7	IARC 2B EU Category 2 carcinogen NTP “Reasonably anticipated to be a human carcinogen”	X		
Solvent Yellow 3 97-56-3	IARC 2B EU Category 1B carcinogen NTP “Reasonably anticipated to be a human carcinogen”	X	*o*-Toluidine (substance is also an EU22)	
Solvent Orange 3 495-54-5				Solvent Orange 3 is toxicologically equivalent to Basic Orange 2, which was considered to have carcinogenic potential in a separate

Substance Names and CAS RN	Classification for carcinogenicity[b]	Evidence of carcinogenicity from animal studies and/or human epidemiology	Release of EU22 aromatic amine by azo bond cleavage	Read-across
				screening assessment for Certain Azo Basic Dyes (Environment Canada and Health Canada, 2014a).
Solvent Yellow 77 2832-40-8	EU Category 2 carcinogen	X		
Solvent Yellow 14 Sudan I 842-07-9	EU Category 2 carcinogen	X		Read-across (Sudan Dyes) (See section 7.2.2)
Solvent Orange 2 Oil Orange SS 2646-17-5	IARC 2B EU Category 2 carcinogen	X	*o*-Toluidine	Read-across (Sudan Dyes) (See section 7.2.2)
Solvent Red 1 1229-55-6	EU Category 1B carcinogen		*o*-Anisidine	Read-across (Sudan Dyes) (See section 7.2.2)
Solvent Red 24 Sudan IV 85-83-6	EU Category 2 carcinogen		*o*-Toluidine	Read-across (Sudan Dyes) (See section 7.2.2)
Solvent Red 19[a] 6368-72-5			*p*-aminoazobenzene	
21519-06-2[a]			*p*-aminoazobenzene	

[a] There is uncertainty with respect to the extent of azo bond reduction and the actual metabolites released *in vivo,* in particular for a disazo substance with a postulated cleavage product that contains an azo bond (See Section 7.2.5 Uncertainty in Health Effects Assessment).

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