The Chemicals Management Plan Substance Groupings Initiative

Aromatic Azo- and Benzidine-Based Substances

Draft Technical Background Document

Environment Canada

Health Canada

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INTRODUCTION

The *Canadian Environmental Protection Act, 1999* (CEPA 1999) required the Minister of the Environment and the Minister of Health to categorize all substances on the Domestic Substances List (DSL) to identify those 1) that are persistent and/or bioaccumulative (based on the *Persistence and Bioaccumulation Regulations* of CEPA 1999) and inherently toxic to humans or other organisms or 2) that present or may present, to individuals in Canada, the greatest potential for exposure. CEPA 1999 further requires the Ministers to conduct screening assessments of substances that have met the categorization criteria set out in the Act to determine whether these substances present or may present a risk to the environment or to human health.

Based on the information obtained through the categorization process, which was completed in 2006, the Ministers identified a number of substances as high priorities for action. These include substances that

- met all of the ecological categorization criteria, including persistence (P), bioaccumulation potential (B) and inherent toxicity to aquatic organisms (iT), and were believed to be in commerce in Canada; and/or
- met the categorization criteria for greatest potential for exposure (GPE) or intermediate potential for exposure (IPE) and had been identified as posing a high hazard to human health based on classifications by other national or international agencies for carcinogenicity, genotoxicity, developmental toxicity or reproductive toxicity.

Out of the approximately 23 000 substances subject to categorization, about 4300 were identified as requiring screening assessments. These substances became a focus for further work under Canada's Chemicals Management Plan (CMP), launched on December 8, 2006. The first phase of the CMP included a number of initiatives, including the Challenge, which addressed approximately 200 high-priority substances identified during categorization. Of the 4300 substances identified as priorities for further action, approximately 1100 have been addressed, and 3200 remain to be addressed.

The Substance Groupings Initiative, identifying approximately 500 substances in nine groups as the next round of priorities, is one of the key initiatives of the CMP moving forward. This initiative began with a Notice of Intent for the aromatic azo- and benzidine-based substances group, published on June 5, 2010 (Canada 2010). On October 8, 2011, an announcement of planned actions to assess and manage, where appropriate, the risk posed by certain substances to the health of Canadians and the environment, that applies to this Substance Grouping and to eight additional groups of substances, was published in the Canada Gazette, Part I, Volume 145, No. 41 (Canada 2011b).

The aromatic azo- and benzidine-based substances group consists of 358 substances that were identified as priorities for action through the categorization process, as they met the Government

of Canada categorization criteria under section 73 of CEPA 1999 (Canada 1999) and/or were considered as priority substances under the CMP based on other ecological and/or human health concerns (Environment Canada 2007a; Health Canada 2009). 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 substances (aromatic amines or benzidines) that are known or likely to be carcinogenic and/or genotoxic. Many of these substances have common structural features and similar functional uses as colourants, which are used in multiple sectors. Therefore, addressing these substances as a group supports a consistent scientific approach to assessment. Establishing subgroups within this group based on characteristics such as chemical structures, use patterns and physical-chemical properties accounts for variability within this Substance Grouping and allows for subgroup-specific approaches in the conduct of screening assessments. Screening assessments focus on information critical to determining whether substances within each subgroup 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.

This document outlines the subgrouping approach, which has been subject to external peer consultation, including review by Dr. Harold Freeman (North Carolina State University) and Dr. David Lai (US Environmental Protection Agency). The subgrouping approach was also subject to consultation by technical experts and other stakeholders via a multi-stakeholder technical consultation held on March 20, 2012, in Ottawa, Canada. This document also summarizes the overarching technical knowledge related to substances in this Substance Grouping and is intended as a supporting document for subsequent development of screening assessments moving forward.

The list of substances included in the aromatic azo- and benzidine-based substances group is found in Appendix I.

Literature searches were conducted in the preparation of the draft Technical Background Document on the aromatic azo- and benzidine-based substances group, up to May 2011 for the human health and ecological sections of the document.

This draft Technical Background Document was prepared by staff in the Existing Substances Programs at Health Canada and Environment Canada and incorporates input from other programs within these departments.

BACKGROUND

Some azo dyes based on benzidine, benzidine derivatives, and aromatic amines 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 substances (aromatic amines or benzidines) that are known or likely to be carcinogenic and/or genotoxic. The international context on aromatic azo- and benzidine-based substances is summarized in this section. Other jurisdictions such as the European Union (EU) and the United States have taken or proposed actions to manage the risks posed by some of these substances. Azo dyes based on benzidine and aromatic amine classified as carcinogenic in the European Union are no longer manufactured within EU member countries (Klaschka 1994). In the United States, the majority of manufacturers began phasing out the use of benzidine-based dyes in the mid-1970s (IARC 2010a). While some global phase-out of restricted azo colourants has been noted (Øllgaard et al. 1998), it has been noted that restricted azo colourants may be present in some manufactured items and products manufactured in Asia, Eastern Europe and South America (Øllgaard et al. 1998). As imported manufactured items and products containing azo colourants are potential sources of exposure in Canada, historical background and information on how these colourants are managed internationally are considered important to the Canadian context.

European Union

The first EU country to enact legislation on the use of certain azo colourants was Germany, in the form of amendments to the German Consumer Goods Ordinance in 1994 (Germany 1997). This legislation placed restrictions on the use of certain azo colourants in consumer products intended for more than temporary contact with the skin (ETAD 2002). The MAK Commission of the German Research Foundation (Deutsche Forschungsgemeinschaft) has recommended that azo colourants be classified by component aromatic amines known or suspected to be carcinogenic (DFG 2007).

Following the German legislation, several other member states of the EU enacted similar legislation (ETAD 2002). These legislations were harmonized in 2002 (ETAD 2002), when EU Directive 76/769/EEC (1976) (EU 1976) was amended by Directive 2002/61/EC (EU 2002) to restrict the placing on the market of certain azo dyes across EU member states. This Directive was repealed and replaced by the EU's Commission Regulation (EC) No 1907/2006 (EU 2006), later amended to Commission Regulation (EC) 552/2009 of June 22, 2009, in Annex XVII of the REACH database, which describes conditions of restrictions on azo dyes (EU 2009a). The restrictions apply to azo dyes that, by reductive cleavage of one or more azo groups, may release one or more of the aromatic amines listed in Appendix 8 in detectable concentrations (i.e., above 30 parts per million [ppm]) in the finished articles or in the dyed parts thereof, according to the

testing methods outlined in Appendix 10. The restrictions apply to textile and leather articles that may come into direct and prolonged contact with the human skin or oral cavity, such as:

- clothing, bedding, towels, hairpieces, wigs, hats, diapers and other sanitary items, sleeping bags;
- footwear, gloves, wristwatch straps, handbags, purses/wallets, briefcases, chair covers, purses worn around the neck;
- textile or leather toys and toys that include textile or leather garments; and
- yarn and fabrics intended for use by the final consumer (EU 2009a).

Due to the large number of existing azo colourants, periodic withdrawals from the market of some older azo colourants, continued development of new azo colourants, as well as the confidentiality of the chemical composition of some manufactured colourant formulations, derivation and maintenance of a comprehensive list of all potential azo colourants that may split off to form at least one of the 22 EU aromatic amines is not considered feasible (DTI 1998). Directive 2002/61/EC (EU 2002), which was later replaced by Annex XVII in the EU's Commission Regulation (EC) No 1907/2006 (EU 2006), did not specifically contain restrictions on azo pigments (ETAD 2008). However, despite the generally very low solubility of azo pigments, under the prescribed test conditions of Article 2a of Directive 2002/61/EC, some azo pigments may be sufficiently soluble to release a listed aromatic amine above the detection limit of 30 ppm (ETAD 2008).

In addition, Regulation (EC) No 1223/2009 of the European Parliament and of the Council of November 30, 2009, on cosmetic products, establishes rules to be complied with by any cosmetic product made available on the market, in order to ensure the functioning of the internal market and a high level of protection of human health (EU 2009b). Annex II of the regulation lists substances that are restricted in cosmetic products, Annex III lists substances that cosmetic products must not contain except subject to the restrictions laid down and Annex IV lists colourants allowed in cosmetic products (EU 2009b). Among the 358 substances in this Substance Grouping, 30 substances are listed on Annex II and 19 substances are listed on Annex IV.

Food colourants are regulated by the European Commission, and approved food colourants are listed in Council Directive 94/36/EC in Europe (EU 1994). Furthermore, Regulation (EC) No 1333/2008 of the European Parliament and of the Council of December 16, 2008, on food additives lays down rules on additives used in foods with a view to ensuring the effective functioning of the internal market while ensuring a high level of protection of human health, the protection of consumer interests and the maintenance of fair practices in the food trade, taking into account, where appropriate, the protection of the environment (EU 2008). Article 24 of the regulation describes the labelling requirement for foods containing certain food colours. Annex V of article 24 contains a list of food colours for which the following additional information must be provided on the label: "may have an adverse effect on activity and attention in children"

(EU 2008). Foods in which the colours have been used for the purposes of health or other marking on meat products or for stamping or decorative colouring on eggshells are exempt from this additional labelling requirement (EU 2008).

United States

A significant new use rule (SNUR) under section 5(a)(2) of the *Toxic Substances Control Act* (TSCA) was developed for benzidine-based chemical substances in 1996. This requires persons to notify the US EPA at least 90 days before commencing the manufacture, import or processing of certain benzidine-based chemical substances for any significant new use as described in this rule. This SNUR lists 24 substances (US EPA 1996). In August 2010, the US EPA released an action plan that addresses 48 dyes derived from benzidine and its derivatives. Under that action plan, the US EPA is taking the following actions (US EPA 2010):

1) Initiate rulemaking to add four benzidine-based dyes to an existing TSCA section 5(a)(2) SNUR for benzidine-based substances at Title 40 of the Code of Federal Regulations (CFR), section 721.1660.

2) Initiate rulemaking to establish a new TSCA section 5(a)(2) SNUR for benzidine derivative–based dyes, including 44 specific such dyes.

3) Consider proposing to eliminate the article exemption applied to SNURs to address potential concerns for exposure to these dyes on imported finished textiles.

4) Consider initiating action under TSCA section 6 if the US EPA learns that these dyes are present in imported finished textiles. The US EPA has proposed eliminating the article exemption as part of the proposed SNUR for benzidine-based substances issued on March 20, 2012 (US EPA 2012).

5) Consider additional regulatory action if the US EPA determines that there are other ongoing uses for these dyes and needs to obtain information necessary to determine whether those uses present concerns that need to be addressed.

In March 2012, the US EPA issued a proposed SNUR to add nine benzidine-based substances (the four noted in the action plan plus five others found on the US EPA's Confidential Business Information Inventory of Chemicals) to the existing SNUR for benzidine-based substances. The US EPA will accept comments on the proposal until 60 days after publication in the Federal Register (US EPA 2012).

In the United States, food colourants are regulated by the Food and Drug Administration under Title 21 of the CFR. Colourants are classified as either "certifiable" or "exempt from certification" and are regulated under Parts 74 and 73 of Title 21 of the CFR, respectively. Certifiable colours are synthetic, and manufacturers must provide samples of each batch of

synthetic colour to the FDA for analysis to ensure safety and quality (similar to the certification process that exists in Canada). Once certified, these colours are designated Food, Drugs and Cosmetics (FD&C). Colours exempt from certification are those derived from natural sources (mainly plant, sometimes animal/mineral); they may be naturally extracted from the source material or synthesized in the laboratory as a nature-identical.

Other Countries

India has published legislation prohibiting the handling of 112 azo- and benzidine-based dyes. Since 1993, the Government of India has prohibited the handling of 42 benzidine-based dyes (CCRI 2007). The Ministry of Environment and Forests further prohibited the handling of 70 more azo dyes in 1997 (India 1997). "Handling," as defined under the *Environment (Protection) Act, 1986*, includes manufacture, processing, treatment, package, storage, transportation, use, collection, destruction, conversion, offering for sale, transfer or the like of such substances.

In Japan, voluntary industry standards for ensuring the safety of textile products were published on March 29, 2012. In addition, the Minister of Economy, Trade, and Industry has requested that the Ministry of Health, Labour, and Welfare, which is responsible for the *Act on Control of Household Products Containing Harmful Substances*, consider regulating textile products that use azo dyestuffs that may break down into toxic aromatic amines (METI 2012).

In Australia and New Zealand, Standard 1.3.1 of the Australia New Zealand Food Standards Code lists colours approved for use in foods (ANZFSC 2011).

Available information indicates that other countries, such as China, Syria and Turkey, have enacted or are in the process of enacting legislation or other risk management actions on certain azo- and benzidine-based substances (DTI 1998; Chouchani Cherfane 2006; China 2010).

AZO COLOURANTS

Most substances in the aromatic azo- and benzidine-based substances group are used primarily for coloration and belong to the general category of "azo colourants," which account for 60–80% of all organic colourants due to their relatively simple synthesis, good technical performance and wide spectrum of colours (Øllgaard et al. 1998; Püntener and Page 2004). Colourants may be classified according to the chromophoric (i.e., colour-producing) group present, such as the azo chromophore (i.e., azo bond, -N=N-) in the case of azo colourants.

Azo colourants produce colour because they selectively reflect, transmit or scatter light in the visible spectrum (i.e., 400–750 nm), contain one or more chromophoric groups (i.e., azo bonds), have a conjugated system of electrons and exhibit stability due to resonance of electrons (Abrahart 1977; Øllgaard et al. 1998). In addition to containing this chromophoric system, azo colourants may also possess functional groups known as "auxochromes" (i.e., "colour helpers"), such as carboxylic acid, sulfonic acid, amino groups and hydroxyl groups. While auxochromes do not produce colour, they can alter both the intensity and the wavelength of absorbed light, and they may also be used to influence solubility (Abrahart 1977). Therefore, the presence of these substituents influences the physical-chemical properties and performance characteristics of the colourant (DTI 1998).

The term "colourant" encompasses both dyes and pigments (Püntener and Page 2004). By definition, dyes are soluble in the application medium (mainly water-based solvents, but also other non-aqueous solvents) and lose their crystalline or particulate structure during the application process. In contrast, pigments generally exhibit very low solubility in the substrate in which they are incorporated and exist as solid particles or crystallites (Herbst and Hunger 2004). Pigments, especially those of coarser particle size, may opacify the application substrate (Müller and Poth 2006).

Nomenclature

Some commercial azo colourants are classified according to the Colour Index (C.I.) system of nomenclature (Hunger et al. 2005). Each colourant is given a C.I. Generic Name, composed of a C.I. Classification and a C.I. Serial Number. The C.I. Classification provides the application class and the hue of the colourant. The chronological order in which a colourant is registered with the C.I. determines its C.I. Serial Number. Those C.I. Serial Numbers containing a colon indicate that the colourant differs slightly from the parent C.I. Generic Name, usually due to minor differences in chemistry, but also possibly due to different crystal modifications in the case of pigments (SDC 2011).

Each colourant also has a corresponding five-digit C.I. Constitution Number that uniquely identifies its chemical structure. Azo colourants comprise C.I. Constitution Numbers ranging from 11 000 to 36 999, depending on the number of azo bonds that the colourant contains. For

example, a monoazo colourant has one azo bond, a disazo colourant has two azo bonds, a trisazo colourant has three azo bonds, etc. (Øllgaard et al. 1998; Hunger et al. 2005):

- C.I. 11 000 – 19 999: Monoazo
- C.I. 20 000 – 29 999: Disazo
- C.I. 30 000 – 34 999: Trisazo
- C.I. 35 000 – 36 999: Four or more azo bonds

As an example of the C.I. system of nomenclature, C.I. Direct Yellow 12 (Chemical Abstracts Service Registry Number[1] [CAS RN] 2870-32-8) has the C.I. Classification "Direct Yellow" and the C.I. Serial Number "12." The C.I. Classification for this substance indicates that it belongs to the direct dye application class and has a yellow hue. This substance has a C.I. Constitution Number of "C.I. 24895," which indicates that it is a disazo colourant (i.e., between C.I. 20 000 and C.I. 29 999).

Azoic substances[2] belong to a separate group composed of the C.I. Constitution Numbers ranging from C.I. 37 000 to C.I. 39 999. Furthermore, since 1997, chemical structures newly registered with the C.I. have been assigned six-digit C.I. Constitution Numbers due to the large number of colourants in certain areas of the C.I. Constitution Number scale, particularly in the monoazo colourant area (SDC 2011). Within the aromatic azo- and benzidine-based substances group, eight substances possess a six-digit C.I. Constitution Number, namely, C.I. Acid Red 119 (CAS RN 70210-06-9), C.I. Solvent Red 33 (CAS RN 73507-36-5), C.I. Reactive Blue 225 (CAS RN 108624-00-6), C.I. Disperse Violet 52 / C.I. Disperse Red 179 (CAS RN 16586-42-8), C.I. Disperse Orange 61 (CAS RN 55281-26-0), C.I. Disperse Red 338 (CAS RN 63134-15-6), C.I. Disperse Blue 125 (CAS RN 66693-26-3) and C.I. Disperse Blue 287 (CAS RN 72828-64-9).

Synthesis

Azo colourants are strictly anthropogenic in origin; however, some substances containing an azoxy (i.e., *N*-oxide azo) group may also be naturally produced (Hunger et al. 2005).

Synthesis of azo colourants is performed in two steps: diazotization of a primary aromatic amine followed by coupling. The first step is typically performed in the presence of nitrous acid

[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.

[2] Azoic dyes, often known as naphthol dyes, are formed through the application of a soluble naphthol coupling component to a substrate followed by a solution of a diazo component (i.e., "diazo salt") to generate an insoluble "dye" within the fibre (Wilson 2004).

(HNO_2), which has been generated *in situ* with hydrochloric acid (HCl) and sodium nitrite ($NaNO_3$). A primary aromatic amine is used to provide a conjugated system of electrons adjacent to the azo bond that is necessary for colour formation. This primary aromatic amine, known as the "diazo component," belongs to one of three groups: 1) aniline and substituted anilines, 2) naphthylamines and naphthylaminesulfonic acids and 3) diamines (Hunger 2003; IARC 2010a).

A diazonium salt may be formed through steps 1 to 4, as outlined below (refer to Figure 1):

1) A primary aromatic amine is nitrosated by nitrous acid to generate an *N*-nitroso compound.
2) The *N*-nitroso compound tautomerizes[3] to a diazo hydroxide.
3) The hydroxyl group (-OH) of the diazo hydroxide is subsequently protonated.
4) Water is eliminated to generate a resonance-stabilized diazonium salt.

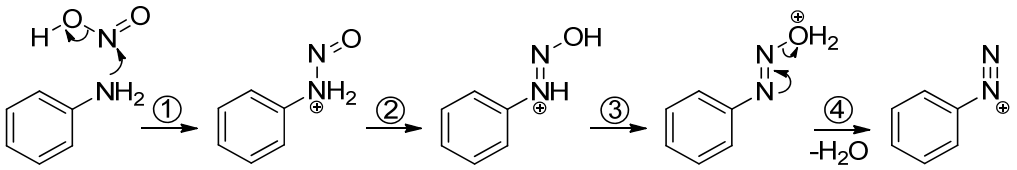


Figure 1: General mechanism of a diazotization reaction of a primary aromatic amine.

The formed diazonium salt is unstable at room temperature, and therefore a low temperature is required for its existence. It is a weak electrophile and will react only with highly electron-rich species, such as amino, hydroxyl and methoxy groups. These highly electron-rich species are known as the "coupling component" (Hunger 2003) (refer to Figure 2).

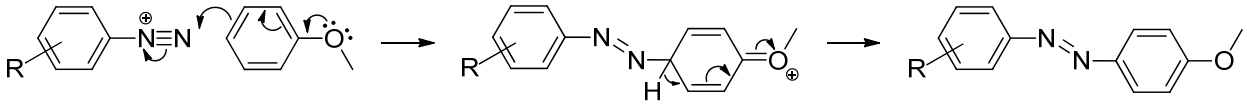


Figure 2: Example of an azo coupling reaction between a diazonium salt and a coupling component (http://www.ecompound.com/Reaction%20reference/reaction_index.htm).

The nitrogen atoms of the azo bond (-N=N-) are linked to sp^2-hybridized carbon atoms, of which at least one is a member of an aromatic carbocycle, usually a benzene or naphthalene derivative.

[3] Tautomerization is delocalization of electrons resulting in mobilization of an atom or a group of atoms or leading to formation of isomeric forms of a substance called "tautomers."

The second carbon atom may be a member of an aryl, heteroaryl or enolizable aliphatic derivative, such as acetoacetic acid (Hunger et al. 2005). Azo colourants containing only aromatic groups are known as carbocyclic azo colourants, while those containing at least one heterocyclic group are known as heterocyclic azo colourants.

The complete reaction of diazo and coupling components may require dispersing agents or emulsifiers if issues of solubility are encountered. Multiple coupling reactions will produce a substance with several azo bonds (i.e., polyazo substance) and will extend the length of the conjugated system, causing a bathochromic shift[4] in colour (i.e., darker colour) (IARC 2010a). Carbocyclic azo dyes provide a wide range of colours, representing most commercial azo dyes. "Azoic dyes" also belong to this class; however, these dyes are formed in the fibre pores only during the application process (Hunger 2003). The majority of industrial azo dyes contain anilines, extended anilines or fused ring amines as components (Øllgaard et al. 1998).

Commercial products of synthesized azo colourants can contain impurities such as residues of aromatic amines used as intermediates in the manufacturing process. Breakdown products in stored commercial products may also be introduced through thermal or photochemical degradation of the azo colourants over time (Øllgaard et al. 1998).

Tautomerization

Azo colourants may undergo tautomerization between the azo and hydrazone forms. This tautomerization is important commercially, as each tautomeric form may differ in colour, performance properties, toxicological profile and tinctorial strength.[5] An example of azo–hydrazone tautomerism is presented in Figure 3.

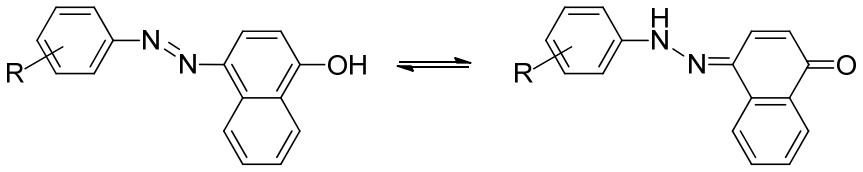


Figure 3: An example of the tautomerism between azo and hydrazone forms (Hunger 2003).

As the hydrazone form tends to have greater tinctorial strength, this form is commercially favoured for its cost-effectiveness. The components of the azo colourant may favour one tautomeric form over another. For example, azophenol dyes exist completely in the azo form.

[4] A bathochromic shift (or effect), informally known as a "red shift," is a shift of the spectral band to lower frequencies (i.e., longer wavelengths) owing to the influence of substitution or a change in environment. A bathochromic shift is opposite to a hypsochromic shift (or "blue shift") (IUPAC 1997).

[5] Tinctorial strength (or "colour strength") represents the amount of colourant required to achieve a standard depth of shade.

Hydroxyazo dyes may vary in the proportion of tautomers present, ranging from pure azo form to a mixture of both tautomers to pure hydrazone form (Hunger 2003). Tautomerization of a 3,3′-dichlorobenzidine-based colourant disazo coupled to diethyl malonate is illustrated in Figure 4.

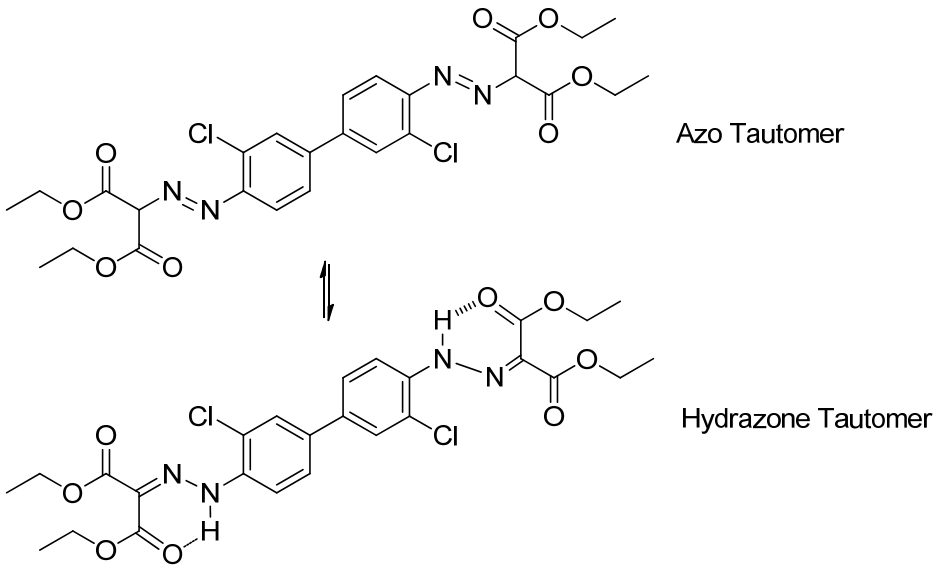


Figure 4: The azo and hydrazone tautomers of a 3,3′-dichlorobenzidine-based colourant disazo coupled to diethyl malonate (De France et al. 1986).

Azo Bond Cleavage

The azo bond is the most labile portion of an azo colourant. This bond may readily undergo cleavage either by biological degradation (biodegradation) and transformation such as enzymatic breakdown (i.e., metabolic cleavage) or abiotic degradation and transformation such as thermal and photochemical degradation and transformation (Øllgaard et al. 1998, Weber and Adams 1995, Shu et al. 1994,). Biological systems contain azoreductases that can enzymatically cleave the azo bond (Bartsch 1981; Chung 1983, 2000; Levine 1991; Chung et al. 1992, 2000; DTI 1998; Xu et al. 2010). Due to the generally very low water solubility of pigments, the corresponding azo bond is usually not available for intracellular enzymatic breakdown (Øllgaard et al. 1998; BfR 2007). However, potential degradation of azo pigments may occur through pathways other than intracellular enzymatic breakdown, for example, through photocleavage with solar radiation (Sperry 1992; Engel et al. 2010).

Certain characteristics of an azo colourant may make it less susceptible to cleavage. It has been noted that sulfonation of azo dyes may inhibit the release of aromatic amines (Øllgaard et al. 1998). The use of dye intermediates capable of metal complexation following incorporation into the dye structure may shield the azo bond from reduction processes (Freeman et al. 1996). In the case of 3,3′-dichlorobenzidine-based colourants that have been disazo coupled to diethyl malonate (e.g., Figure 4), a β-ketone that stabilizes the hydrazone tautomer, reductive cleavage

of the azo bond was not observed, despite the solubility of the test compound (De France et al. 1986).

For the substances in this Grouping Initiative, a number of breakdown products due to azo bond cleavage of one or more azo bonds are theoretically possible. While most of these aromatic amines and benzidine derivatives are not among the CMP priority substances included in this Substance Grouping, some information related to the ecological and human health effects of these substances will be taken into consideration in the assessments where there is sufficient evidence to support azo bond cleavage.

Further details on azo bond cleavage in ecological and human health assessments are found in the respective sections for assessment considerations (i.e., Ecological Assessment Considerations and Human Health Assessment Considerations).

Physical-Chemical Properties and Application Classes

The chemical diversity of azo colourants has allowed for a wide spectrum of colours, particularly in the red portion of the spectrum. Many azo dyes are water soluble, while others are fat soluble. In contrast, azo pigments generally exhibit very low solubility in water and other media (i.e., organic solvents and the substrate to which they are applied), as they are chemically designed to be insoluble in their application medium. This is achieved through a lack of solubilizing groups, incorporation of groups that reduce solubility (e.g., amides) or lake formation of insoluble salts of carboxylic or sulfonic acids (Øllgaard et al. 1998).[6]

Application of dyes and pigments is based on the physical-chemical properties that make them suitable for specific colouring processes. More specifically, an application class provides information on the method and conditions of application required for the colouring of a substrate. For dyes, the application class determines their suitability for different types of substrates. For example, direct dyes are water-soluble anionic dyes that require the presence of electrolytes during the dyeing process. As these direct dyes may be applied directly to cellulosic fibres, major substrates may include rayon and paper. As an additional example, solvent dyes are large hydrophobic molecules that dissolve into their substrates. These solvent dyes are suitable for dyeing inks, plastics, waxes, fat products and mineral oil products (Øllgaard et al. 1998). In certain cases, the same colourant may be classified into more than one application class. Typically, disperse dyes can often be applied as solvent dyes, and vat dyes can sometimes be applied as pigments (SDC 2011).

The effectiveness and efficiency of a dyeing or printing process are dependent on the dye substrate affinity (i.e., "substantivity"). Therefore, dyes are deliberately engineered at the

[6] An azo pigment lake, alternatively referred to as a "toner" or a "lake," is a salt-type pigment formed by precipitating a water-soluble anionic monoazo substance with a metal cation (Herbst and Hunger 2004).

molecular level for the target substrates and intended end use applications. In particular, a dye must have greater affinity for the substrate to which it is applied than for the application medium, and it must display a desired level of permanence under the anticipated end use conditions. Two examples of permanence characteristics are wet-fastness (i.e., stability to fading upon water exposure) and light-fastness (i.e., stability to fading upon exposure to sunlight) (IARC 2010a).

Table 1 provides the general physical-chemical properties of the major application classes relevant to azo colourants in this Substance Grouping. A description of each application class follows. A wide variety of products may be expected to contain azo colourants in this Substance Grouping, including textiles, leather, paints and coatings, inks, cosmetics and personal care products.

Table 1: Physical-chemical properties of the major application classes relevant for azo colourants in this Substance Grouping

Application class	**Vapour pressure**	**K_{ow}**	**Water solubility**	**Ionization**	**Charge**
Acid dyes	Very low[1]	Low	Soluble[2]	Ionic	–
Direct dyes					–
Reactive dyes					–
Basic dyes					+
Disperse dyes		High	Sparingly soluble[3,4]	Non-ionic	Neutral
Mordant dyes					
Solvent dyes					
Pigments		N/A		Variable	Variable

Abbreviations: K_{ow}, octanol–water partition coefficient; N/A, not applicable

1 Azo dyes, where measurements are available, generally have very low vapour pressures (i.e., range of 8.3×10^{-18} to 1.2×10^{-5} Pa) (Baughman and Perenich 1988). The vapour pressures of azo pigments are also anticipated to be very low (i.e., lower than 10^{-11} to 10^{-9} Pa) (Baughman and Perenich 1988).

2 Soluble: > 100 mg/L (ionic dyes) (Øllgaard et al. 1998).

3 Sparingly soluble: < 100 mg/L (pigments and non-ionic dyes) (Øllgaard et al. 1998).

4 In the case of mordant dyes, the descriptor "sparingly soluble" refers to the metal dye complex that has precipitated onto the textile fibre during application, not the pre-application soluble dye itself.

Acid dyes are anionic, water-soluble dyes that generally have lower molecular weights than direct dyes. Principal uses of acid dyes are the dyeing of certain textile fibres, such as nylon, wool, silk and modified acrylic fibres, often through the reaction of one or more sulfonic functional groups that can react with the amide groups of the fibre. To a lesser extent, acid dyes may also be used in paints, inks, plastics and leather (Øllgaard et al. 1998). Food dyes, in most

cases, are structurally similar (e.g., contain multiple sulfonic acid functional groups) to acid dyes and have similar physical-chemical properties (e.g., high water solubility).

Direct dyes are anionic, water-soluble dyes. They are narrow, flat molecules capable of aligning with flat cellulose molecules via van der Waals forces. Direct dyes are bound to fibres through deposition in cavities. Principal uses of direct dyes are the continuous colouring of paper, cellulosic textile fibres (e.g., cotton and rayon) and leather (Øllgaard et al. 1998).

Reactive dyes are chemically similar to direct dyes except that they contain reactive groups (ETAD 1995). These reactive groups covalently bond to hydroxyl, sulfhydryl and amino groups in fibres (Øllgaard et al. 1998). A principal use of reactive dyes is the dyeing of cellulosic textile fibres (e.g., cotton and rayon), where the dye reacts with the hydroxyl groups of the fibre (ETAD 1995).

Basic dyes are cationic, water-soluble dyes that are generally used on certain textile fibres that are negatively charged polymers (e.g., acrylic, modacrylic, modified polyester). Principal uses of basic dyes are the dyeing of polyacrylonitrile fibres and paper.

Disperse dyes are non-ionic, largely water-insoluble dyes typically sold as dispersible powders or pastes. They are typically applied to synthetic fibres from aqueous dispersions under slightly acidic conditions. The dye molecules adsorb onto the fibre surface, then migrate into the interior as the temperature is raised. Principal uses of disperse dyes are the dyeing of polyester, polyester blends, cellulose acetate and nylon (ETAD 1995).

Mordant dyes are non-ionic dyes that, in combination with a mordant, form an insoluble metal dye complex that precipitates on the textile fibre. A mordant is a metal compound, commonly including chromium, aluminum, copper or iron. Principal uses of mordant dyes are the colouring of wool, leather, furs and anodized aluminum (Øllgaard et al. 1998).

Solvent dyes are non-ionic dyes that are sparingly soluble in water and tend to have higher molecular weights than disperse dyes. Principal uses of solvent dyes are the coloration of inks, plastics, waxes, fat products and mineral oil products (Øllgaard et al. 1998).

Pigments generally exhibit very low solubility in water, maintaining their crystalline structure throughout application (Herbst and Hunger 2004). While dyes exist in molecular form, pigments exist as solid particles. Principal uses of pigments are the coloration of graphic printing inks, paints and coatings, plastics and fibres. Azo pigments are similar to disperse, solvent and mordant dyes with respect to their large molecular size, very low solubility in water and hydrophobicity. However, a major distinction for azo pigments is their generally low solubility in organic solvents as well (Øllgaard et al. 1998). As such, azo pigments remain virtually in the solid state during processing and application to a substrate (Clarke and Anliker 1980). The density of azo pigments tends to be higher than that of water at standard temperatures (i.e., > 1000 kg/m^3).

AROMATIC AMINES

Aromatic amines are widely used chemicals. In addition to being intermediates for the synthesis of azo colourants, they may be used to synthesize pesticides, pharmaceuticals, explosives, rubber, epoxy polymers and polyurethane. Aromatic amines may also be used as antioxidants in elastomers. Aromatic amines may be generated through the combustion of organic materials, including in emissions of tobacco smoke (Platzek 2010). Aromatic amines may be found naturally in plants such as corn grains, beans and tea (Australia 2012).

There are 23 aromatic amines in this Substance Grouping, five of which are benzidine derivatives (refer to Tables I-3 and I-14 in Appendix I). They fall into one of two chemical structural types: monocyclic and bicyclic. Respective examples for these two types are 4-methylbenzenamine (CAS RN 106-49-0) and 2-naphthalenamine (CAS RN 91-59-8). *N*-substitution of aromatic amines with aliphatic, aromatic or mixed substituents leads to three categories of aromatic amines: primary, secondary and tertiary. Most aromatic amines in this Substance Grouping are primary aromatic amines, while *N*,*N*,*N*′,*N*′-tetramethylbenzidine (CAS RN 366-29-0) is a tertiary aromatic amine. Various derivatives of aromatic amines exist due to the addition of at least one substituent on the benzene ring of the molecule. For example, 4-chlorobenzenamine (CAS RN 106-47-8) and 4-methylbenzenamine (CAS RN 106-49-0) are both anilines but contain a different substituent at the *para*-position. In addition, there are 10 diamines, including various derivatives of benzidine.

SUBGROUPING

Given the large number of substances with different physical-chemical properties, uses and applications within this Substance Grouping, it was considered appropriate to further divide these substances into subgroups based on their properties. This exercise was applied to 335 substances in this Substance Grouping, excluding 23 aromatic amines, as most of these aromatic amines are potential azo cleavage products of the azo colourants.

The majority (335) of substances were first divided into two categories, named **Chemical Categories**, based on the chemical structure of the substances. The Chemical Categories were established by visual inspection of the molecular structure of each substance (i.e., its SMILES configuration[7]) and consideration of the DSL chemical name. These substances belong to one of the following two chemical categories:

Chemical Category I: Aromatic Azo-based Substances—substances that contain at least one benzene ring (aromatic system) with at least one substituent being an azo bond, as shown in Figure 5. Some of these substances may break down to release aromatic amines. For the purpose of subgrouping into two Chemical Categories, this category excludes substances that contain a benzidine substructure, as described in Chemical Category II below.

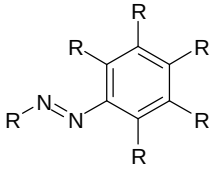
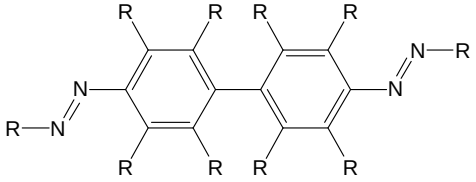


Figure 5: Aromatic azo-based substances.

Chemical Category II: Benzidine-based Substances—substances that contain the benzidine substructure or benzidine derivative substructures, such as those of dimethylbenzidine, dimethoxybenzidine or dichlorobenzidine. The general structure of substances that belong to this Chemical Category is shown in Figure 6.



[7] Simplified Molecular Input Line Entry System: a simple yet comprehensive chemical language in which molecules and reactions can be specified using ASCII characters representing atom and bond symbols; it can be used as a universal identifier for a specific chemical structure.

Figure 6: Benzidine-based substances.

Within each category, substances were further divided based on their **Application Classes**. The Application Class of a substance is considered to be highly related to its structural similarities (e.g., the presence of solubilizing functional groups), its physical-chemical properties and its potential commercial uses. The C.I. Classification was used as the base data source for this step. For those substances that have not been registered with the C.I., an Application Class was inferred, where appropriate, based on other available information, such as DSL use codes[8] and information from the public domain regarding uses and substructures associated with certain Application Classes (Hunger 2003; Herbst and Hunger 2004). For those substances whose use data did not correspond to any available Application Class or whose Application Classes were not identifiable, an "Unknowns" group was formed. In total, 10 Application Classes were identified, as follows: Pigments, Solvent Dyes, Disperse Dyes, Acid Dyes, Direct Dyes, Reactive Dyes, Basic Dyes, Mordant Dyes, Food Dyes and Unknowns.

For aromatic azo-based substances (i.e., non-benzidine-based azo substances), further division was conducted based on the number of azo bonds within each application class, as follows:

1) Monoazo substances: Substances containing one azo bond
2) Disazo substances: Substances containing two azo bonds
3) Polyazo substances: Substances containing three or more azo bonds

Subgroups were established based on both Chemical Category and Application Class. For example, the subgroup "Benzidine-based Pigments" contains substances that belong to Chemical Category II: Benzidine-based Substances and to Application Class "Pigments" and consists of seven substances. A subgroup may also contain more than one Application Class, as in the case of the subgroup "Benzidine-Based Dyes," which contains substances that belong to Chemical Category II: Benzidine-based Substances and also to several Application Classes (i.e., Acid Dyes, Direct Dyes, Basic Dyes and Unknowns). Aromatic amines and benzidine derivatives will be considered separately, taking into consideration the association with their corresponding dyes and/or pigments, as appropriate. A description of each subgroup is summarized in Table 2, along with the number of substances belonging to each subgroup.

While there may be cases where a substance could belong to more than one subgroup, depending on its intended applications, to facilitate assessment, each substance was placed into only one subgroup. For example, there are five substances that have been identified as potential food dyes (NCI 2007; CII 2011). Out of these five substances (CAS RNs 1934-21-0, 3761-53-3, 915-67-3, 2611-82-7 and 106028-58-4), only one substance was placed in the Azo Food Dyes subgroup (CAS RN 106028-58-4, C.I. Food Black 2), as no other Application Class was identified for this

[8] The DSL use codes indicate the general uses reported for each substance within industry submissions for the DSL compilation period of 1984–1986.

substance. The other four substances were placed in the Azo Acid Dyes subgroup, as they are also known to be applied as acid dyes for textiles.

Table 2: Subgroup description

Chemical Category	**Subgroup (number of substances)**	**Application Class**	**Azo substructure (number of substances)**
I. Aromatic Azo-based Substances	Monoazo Pigments (33)	Pigments	Monoazo- (33)
	Azo Solvent Dyes (22)	Solvent Dyes	Monoazo- (15); Disazo- (7)
	Azo Disperse Dyes (73)	Disperse Dyes	Monoazo- (62); Disazo- (11)
	Azo Acid Dyes (52)	Acid Dyes	Monoazo- (21); Disazo- (20); Polyazo- (11)
	Azo Direct Dyes (61)	Direct Dyes	Monoazo- (5); Disazo- (37); Polyazo- (19)
	Azo Reactive Dyes (8)	Reactive Dyes	Monoazo- (4); Disazo- (4)
	Azo Basic Dyes (33)	Basic Dyes	Monoazo- (28); Disazo- (5)
	Azo Mordant Dyes (2)	Mordant Dyes	Monoazo- (2)
	Azo Food Dyes (1)	Food Dyes	Disazo- (1)
	Unknowns (4)	Unknowns	Monoazo- (3); Disazo- (1)
II. Benzidine-based Substances	Benzidine-based Pigments (7)	Pigments	N/A
	Benzidine-based Dyes (39)	Acid Dyes Direct Dyes Basic Dyes Unknowns	N/A

Abbreviation: N/A, not applicable

Substances within each subgroup may be further divided into **Structurally Related Groups** based on more detailed structural similarities. Limited scientific information is available for many of the substances in this Substance Grouping, and identification of structurally related substances supports use of structure–activity relationship (SAR) (i.e., read-across) approaches across similar substances. In the absence of empirical data, analogues (i.e., chemically similar substances) may be used to inform assessments. Additionally, consistent with international best practices, modelled data may be generated through the use of quantitative structure–activity relationships, or (Q)SARs.

The physical-chemical properties of some dyes and pigments are not amenable to model prediction, because most (Q)SAR models are not generally designed to handle particles or substances that tend to aggregate and do not exert standard molecular characteristics. Many substances within this Substance Grouping include pigments and non-ionic dyes as well as ionizing substances, such as acid, direct, basic and reactive dyes (Environment Canada 2000). Therefore, the applicability of (Q)SAR models to dyes and pigments in this Substance Grouping will be determined on a case-by-case basis.

HUMAN HEALTH ASSESSMENT CONSIDERATIONS

Exposure Assessment

Based on available information, a key source of exposure of the general population for the majority of substances in this Substance Grouping is considered to be use of, or contact with, certain manufactured articles and products. For each Application Class, generic exposure scenarios for sentinel product categories will be developed to inform the screening assessments based on either CAS RN-specific information or information relevant to an Application Class. Use of information relevant to an Application Class supports a consistent assessment approach within the Application Class.

Concentration ranges of substances within this Substance Grouping in products will be obtained from available information in the literature and from information submitted in response to a section 71 survey issued under CEPA 1999 (Canada 2011a), as appropriate. Preliminary sentinel product categories, concentration ranges and sources of information have been summarized in Tables 4–6, presented by route of exposure (i.e., oral, dermal, inhalation). Additional sentinel product categories may be identified moving forward.

Based on the information available, major uses of colourants identified across several application classes were textiles, leather and paints and coatings. For example, for textile products, a child care product (i.e., a baby jumper) and personal apparel were identified as sentinel product categories for potential exposure by the dermal route. For exposure by the oral route, textile-based toys were identified as a sentinel product category for children less than 4 years of age as a result of mouthing behaviours. For leather products, leather apparel (i.e., jacket, trousers, footwear), leather furniture and leather-based toys were identified as sentinel product categories for potential exposure by the dermal route. In the case of paints and coatings, children's poster paint, finger paint and a painted toy car were selected as sentinel product categories due to the likelihood of dermal and oral exposure of children.

In addition to the products summarized in Tables 4–6, the use of pigments in permanent tattoo inks injected into the dermis is a potential source of exposure. Based on the information available, a typical in-use pigment concentration would be 2.53 mg pigment per square centimetre *ex vivo* human or pig tattooed skin (Engel et al. 2008).

Table 4: Type of sentinel products considered for the oral exposure route

Product	Concentration range of substance (% by weight)	References for concentration range
Personal Care Products and Cosmetics		
Lipstick	5–8	CNS 2011
Oral hygiene products (e.g. mouthwash, toothpaste)	<0.1–0.3[1]	CNS 2011
Consumer Products		
Ball pen ink	1–5	Scott and Moore 2000
Finger paint	3–60	IARC 2010b
Marker pen ink	1–5	Scott and Moore 2000
Painted toy car	3–60	IARC 2010b
Textile toy	1	BfR 2007
	0.5–3	Øllgaard et al. 1998
Writing ink (child)	1–5	Scott and Moore 2000

[1] Concentration is notified in the following ranges under CNS: <0.1, 0.1-0.3, 0.3-1, 1-3, 3-10, 10-30, and 30-100% (CNS 2011).

Table 5: Types of sentinel products considered for the dermal exposure route

Product	Concentration range of substance (% by weight)[1]	References for concentration range
Personal Care Products and Cosmetics		
Antiwrinkle preparation	<0.1–10	CNS 2011
Baby body lotion[2]	<0.1–3	CNS 2011
Bath salts[3]	<0.1–30	CNS 2011
Face makeup (blush, foundation)	<0.1–30	CNS 2011
Deodorant	<0.1–30	CNS 2011
Eye shadow[4]	<0.1–30	CNS 2011
Fragrance	<0.1–30	CNS 2011
Hair bleach	< 0.1	CNS 2011
Hair conditioner	< 0.1–30	CNS 2011
Hair grooming (hair gel, hair spray)	< 0.1–10	CNS 2011
Hair removal	< 0.1–1	CNS 2011
Hair shampoo	< 0.1–100	CNS 2011
Hair waving preparation	< 0.1–30	CNS 2011

Manicure preparation	< 0.1–30	CNS 2011
Massage oil	< 0.1–1	CNS 2011
Hair dye	< 0.1–30	CNS 2011
Shaving cream	< 0.1–3	CNS 2011
Skin cleanser (body)	< 0.1–1	CNS 2011
Skin moisturizer	< 0.1–100	CNS 2011
Tanning preparation	< 0.1–1	CNS 2011
Consumer Products		
Finger paint	3–60	IARC 2010b
Leather apparel (i.e., jacket, trousers, footwear)	2 (dye); 0.1–0.2 (pigment)	Øllgaard et al. 1998
Leather furniture	2 (dye); 0.1–0.2 (pigment)	Øllgaard et al. 1998
Leather toy	2 (dye); 0.1–0.2 (pigment)	Øllgaard et al. 1998
Poster paint (children's)	3–60	IARC 2010b
Tattoo, temporary	1–5	Scott and Moore 2000
Textiles: Baby jumper	1	BfR 2007
	0.5–3	Øllgaard et al. 1998
Textiles: Personal apparel worn by adults	1	BfR 2007
	0.5–3	Øllgaard et al. 1998
Writing ink (adult)	1–5	Scott and Moore 2000

[1] Concentration is notified in the following ranges under CNS: <0.1, 0.1-0.3, 0.3-1, 1-3, 3-10, 10-30, and 30-100% (CNS 2011).
[2] Notified as "Baby Products" in CNS (CNS 2011)
[3] Notified as "Bath Preparation" in CNS (CNS 2011)
[4] Notified as "Eye Makeup" in CNS (CNS 2011)

Table 6: Types of cosmetic and personal care products considered for the inhalation exposure route[1]

Product	**Concentration range of substance (% by weight)**[2]	**References for concentration range**
Fragrance	< 0.1–30	CNS 2011
Hair spray	< 0.1–100	CNS 2011
Temporary hair dye (aerosol form)	< 0.1–30	CNS 2011

[1]As indicated in Baughman and Perenich (1988), azo colourants tend to have very low vapour pressures. As such, inhalation exposure to azo colourants in the gas phase is not typically expected to be a significant route of exposure. However, consumer exposure to azo colourants by the inhalation route of exposure may occur from personal care products and cosmetics that are aerosolized into fine droplets.
[2] Concentration is notified in the following ranges under CNS: <0.1, 0.1-0.3, 0.3-1, 1-3, 3-10, 10-30, and 30-100% (CNS 2011).

Human Health Effects Assessment

When conducting the human health effects assessments of the substances in this Substance Grouping, it is important to highlight several aspects: azo reductive cleavage and the corresponding metabolites (aromatic amines) produced as a consequence, absorption of both parent substances (aromatic azo- and benzidine-based substances) and their metabolites, and human health effects associated with the parent substances and metabolites.

Azo Reductive Cleavage

In vivo, azo reduction of azo- and benzidine-based substances occurs by an enzyme-mediated reaction (Golka et al. 2004). Azoreductases are present and active in the microflora of the gut (Yoshida and Miyakawa 1973; Chung et al. 1978; Hartman et al. 1978; Cerniglia et al. 1982a, b; Bos et al. 1986; Xu et al. 2010) and skin (Platzek et al. 1999; Chen et al. 2005; Stingley et al. 2010). They are also found in mammalian tissues, particularly in liver (Fouts et al. 1957; Walker 1970; Martin and Kennelly 1981; Kennelly et al. 1982).

Azo dye reduction has been described in both anaerobic and aerobic bacteria (Levine 1991; Xu et al. 2007). While aerobic azoreductases have been characterized from both aerobic and anaerobic members of the human microbiome, anaerobic conditions have been considered more common for azo reduction (Stolz 2001; Stingley et al. 2010).

The intestinal microflora plays an important role in azo dye reduction following oral exposure to azo dyes (Bartsch 1981; Chung 1983, 2000; Levine 1991; Chung et al. 1992; Brown and DeVito 1993; Chung 2000; Xu et al. 2010). There is evidence that some molecules require gut flora reduction before they can be further metabolized by the liver (reviewed in Brantom 2005). Studies conducted on dyes derived from benzidine or its derivatives have found that bacterial azoreductase activity is over 100 times more efficient than that of liver azoreductases and may constitute the primary pathway for azo reduction (Martin and Kennelly 1981; Kennelly et al. 1982; Brown and DeVito 1993). Some 45 different species of intestinal bacteria, encompassing a diverse collection that includes strictly anaerobic *Clostridium* species and facultative anaerobic Enterobacteriaceae, have been shown to express azoreductases (Chung and Cerniglia 1992; Moller and Wallin 2000). So far, at least two types of azoreductases have been identified in bacteria: monomeric flavin-free enzymes containing a putative reduced nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) binding motif and polymeric flavin-dependent enzymes (Chen 2006).

Several hundred species of bacteria are expected to be present in human skin (Gao et al. 2007), and a number of these have been shown to possess azoreductase activity. Skin bacteria representing the genera *Staphylococcus*, *Micrococcus*, *Corynebacterium*, *Kocuria*, *Dermacoccus*, *Streptococcus* and *Pseudomonas* were found to be capable of azo reduction; however, they varied in their ability to reduce the azo dyes methyl red (CAS RN 493-52-7) and orange II (CAS

RN 633-96-5). Therefore, the extent of reduction is dependent on both the bacterial species and the structure of the azo substance (Stingley et al. 2010). Azo reduction on the skin surface is particularly relevant, as it could potentially lead to the formation of carcinogenic aromatic amines on skin that are more readily absorbed than their parent substance; these aromatic amines can therefore become systemically available (Platzek et al. 1999).

In the mammalian liver, azo compounds are metabolized by cytosolic and microsomal enzymes, by reductive cleavage to the amines, for example. This is followed by microsomal oxidation and *N*-acetylation or *O*-esterification to form DNA adducts in the liver (Levine 1991; Brown and DeVito 1993). At least three different types of azoreductase activity are found in the liver. They differ with respect to localization, substrate specificity, response to enzyme inducers and sensitivity to oxygen and carbon monoxide. Two of these types of activities are associated with the microsomal fraction and require cytochrome P450, while one activity is located in the cytosol of the liver (Moller and Wallin 2000).

The role of azo reduction in biological activity (e.g., mutagenesis and carcinogenesis) of azo dyes is well established, as outlined in the following section. It is generally recognized that azo colourants whose metabolism can liberate a carcinogenic aromatic amine are potentially carcinogenic. For example, it has been recommended by the German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, also known as the MAK Commission, that such colourants be addressed as though they were classified in the same categories as the corresponding carcinogenic or suspected carcinogenic amines (DFG 2007). Accordingly, when available information demonstrates potential for azo bond cleavage, the genotoxic and/or carcinogenic potential of the resulting aromatic amines will be considered. The potential for azo bond cleavage to the corresponding aromatic amines can be evaluated by the following types of studies:

1) ***In vivo* metabolism studies:** The presence of one or more metabolites in the urine and/or feces of mammalian species orally exposed to the substance provides direct evidence for azo bond cleavage. In evaluating empirical data, it is important to determine that the concentrations of the aromatic amines in the urine and feces (i.e., generated from the azo bond cleavage) are not attributable to levels of aromatic amines present as impurities in the test material. The concentration of the parent compound in the urine and/or feces is to be taken into account in determining the degree of cleavage (Childs and Clayson 1966; Rinde and Troll 1975; Leuschner 1978; Mondino et al. 1978; Lynn et al. 1980; Nony and Bowman 1980; Nony et al. 1980, 1983; Bowman et al. 1982, 1983; Kennelly et al. 1982; Levine et al. 1982; Frantz et al. 1991; Sagelsdorff et al. 1996).

2) ***In vitro* metabolism studies:** The potential for azo bond cleavage can be evaluated following incubation of the substance with intestinal contents, feces, liver extracts from mammalian species or human skin cultures. While most of the studies identified involved incubation with intestinal contents (Hartman et al. 1978; Cerniglia et al. 1982a, b, 1986;

Bos et al. 1986; Rafii et al. 1990; Chung et al. 1992; Xu et al. 2007, 2010), a number of studies involving incubation using liver and skin exist (Martin and Kennelly 1981; Bos et al. 1984; Platzek et al. 1999).

3) **Mutagenicity testing under reductive conditions:** Various modifications have been made to the *Salmonella* mutagenicity assay to enhance the metabolic breakdown of azo dyes specifically. The Prival modification was introduced in 1982 by Prival and Mitchell and incorporated five modifications to the standard assay that were deemed necessary for mutagenic activity. In particular, a cofactor, flavin mononucleotide (FMN), was added to facilitate reductive cleavage of the azo bond and enabled the reduction and S9-mediated metabolic activation to be carried out *in situ*. Another variation to this assay involves first reducing the dye using either rat cecal flora (bacteria with reducing capabilities) or hamster S9 mix containing FMN, extracting the reduction products from the crude mixture and subjecting the reduction products to oxidative metabolism using either rat liver S9 or uninduced hamster liver S9. A number of other methods used to reduce azo dyes prior to their incubation with *Salmonella* bacteria include the use of FMN in cell-free intestinal bacteria and chemical reduction using sodium dithionite. If the *Salmonella* mutagenicity assay yielded positive results only after such conditions were employed, then the potential for the azo dye to cleave into aromatic amines with mutagenic activity could be inferred (reviewed in Freeman et al. 1996).

Absorption

The absorption of bioaccessible intact dyes across biological membranes including the GI tract the skin is largely dependent on two factors: their molecular size and lipid solubility (Brantom 2005). Dyes with greater lipid solubility tend to undergo significant oxidative metabolism in the liver (Combes and Haveland-Smith 1982; reviewed in Levine 1991). Polar, highly water-soluble dyes or those substituted with large, highly charged groups, such as sulfonates, are generally not well absorbed (reviewed in Levine 1991). Studies have indicated that only a few percent of a dose administered orally is excreted in the urine as parent compound (reviewed in Levine 1991). In the large intestine, these substances are exposed to the reductive environment of anaerobic bacteria (Schroder and Johansson 1973; Allan and Roxon 1974). Although parent compounds may not be absorbed, the resulting aromatic amine metabolites may be efficiently absorbed in the intestine (Brown and Devito 1993). Absorption of aromatic amine metabolites, which is also dependent on structure and lipid solubility, and their subsequent metabolism are required for biological activity (e.g., genotoxicity and/or carcinogenicity). For example, highly sulfonated azo dyes undergo azo bond cleavage and subsequently release sulfonated aromatic amines. These aromatic amines are then rapidly absorbed, modified by the liver and excreted in the bile and urine (Parkinson and Brown 1981). Therefore, a decrease in lipid solubility through the presence of sulfonate groups, especially if present in all metabolites, would reduce the potential biological activity of a dye (Brantom 2005). It is also important to point out that, in general, for substances

that are insoluble or have high water or lipid solubility, absorption through oral or dermal exposure routes is limited (Rozman and Klaassen 2001).

Critical Health Effects

It has been shown that genotoxicity and carcinogenicity are critical human health effects for characterization of risk for certain aromatic azo- and benzidine-based substances. Several azo dyes and dye intermediates have been reviewed by the International Agency for Research on Cancer (IARC) and have been categorized into Group 1, 2A or 2B (known, probable and possible human carcinogens, respectively) (reviewed in Brantom 2005; IARC 2010a). In addition, when sufficient evidence for azo bond cleavage has been demonstrated and the aromatic amine produced is one of the 22 aromatic amines listed in the Commission Regulation (EC) 552/2009 of June 22, 2009 (EU 2009a) (see the section on international context– European Union), the potential for carcinogenicity and/or genotoxicity of the parent substance is inferred.

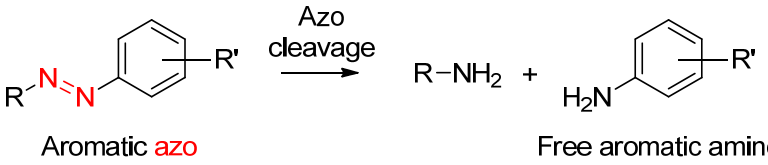
Many genotoxicity and carcinogenicity studies have focused on the health effects of azo dyes, it is recognized that the terms azo "colourants" and azo "dyes" are often used interchangeably in the literature. The potential biological activity of azo dyes often corresponds to the ability of the molecule to generate reactive metabolites (reviewed in Brantom 2005). While some of these metabolites may be of lower hazard than the original dye (Collier et al. 1993), others, such as aromatic amines and free radicals, are potentially carcinogenic (Mason et al. 1977; Chung 1983; Nakayama et al. 1983). It has been estimated that more than 2000 azo colourants have been synthesized, with approximately 450 of them based on aromatic amines that have been classified as carcinogenic (Colour Index 1987; Myslak and Bolt 1988; Platzek et al. 1999). Biological activity is also dependent on both solubility and bioavailability (Golka et al. 2004). SARs of aromatic amines have been analyzed and reviewed in order to predict toxicity and design azo dyes with lower biological activity (Chung and Cerniglia 1992; Brown and DeVito 1993; Freeman et al. 1996; Chung et al. 2000).

Three mechanisms for the metabolic activation of azo dyes have been identified. While they all require some type of metabolic activation to produce reactive electrophilic intermediates that can interact with cellular material, i.e., covalently bind to DNA or ribonucleic acid (RNA), they differ in terms of the sequence of metabolic reactions leading to the reactive intermediates (Brown and DeVito 1993). The three mechanisms are described below:

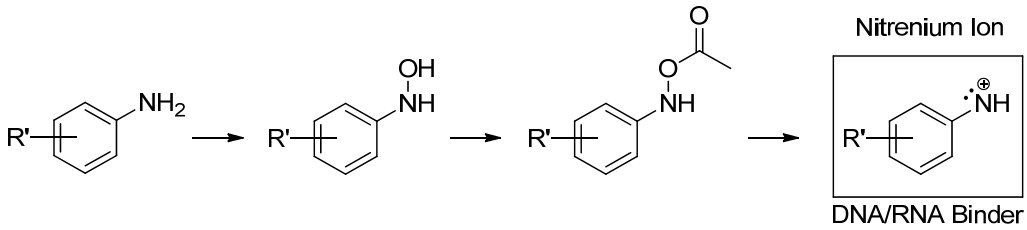
> ***Mechanism I: Aromatic amine(s) released by azo bond cleavage—***The release of aromatic amines by azo bond cleavage is the mechanism by which many azo dyes are converted to reactive intermediates. The reductive cleavage of the azo bond, the most labile portion of an azo molecule, and the subsequent release of the free aromatic amines (Step 1) often determine the potential for azo substances to become biologically active (e.g., mutagenic). There is also evidence that the aromatic amines produced require further metabolic activation (Step 2) for biological activity (e.g., mutagenicity) (Brown

and DeVito 1993). Metabolic activation involves *N*-hydroxylation followed by *O*-acylation, yielding acyloxy amines (Cartwright 1983). These compounds can degrade to form highly reactive nitrenium and carbonium ions, which may readily bind covalently to DNA or RNA.

Step 1. Cleavage of aromatic azo bond and release of the free aromatic amine(s)

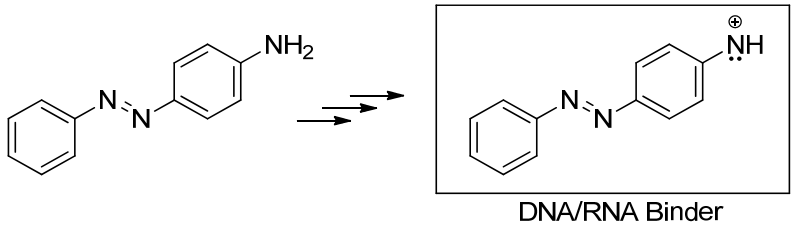


Step 2. Metabolic activation of the aromatic amine and formation of electrophilic reactants



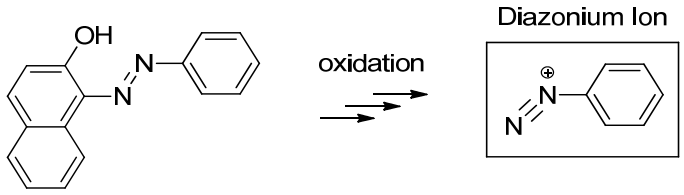
A variation of Mechanism I is the azo reduction of one of multiple azo bonds that are found in certain azo dyes, yielding aminoaryl-substituted monoazo compounds that are biologically active (Brown and DeVito 1993).

Mechanism II: Oxidation of a free aromatic amine group that is part of the azo dye structure—When an azo dye has a free aromatic amine group (or a similar *N*-methylated derivative), azo bond reduction is not always necessary for creation of a reactive intermediate. These aromatic amine groups can undergo a similar metabolic activation process at the nitrogen atom, as shown above in Step 2 of Mechanism I. An electrophilic nitrenium ion can be produced that can subsequently react with DNA and RNA. In such cases, metabolic azo bond reduction is not required and, depending on the azo dye structure, can act as a detoxification mechanism (Brown and DeVito 1993).



Mechanism III: Activation of the azo dyes via direct oxidation of the azo linkage to highly reactive electrophilic diazonium salts—In the liver, certain azo dyes can undergo direct oxidation at the azo bond, without prior azo bond reduction, to generate reactive

intermediates, including diazonium ion, which can further react with cellular DNA, RNA or protein (Brown and DeVito 1993). Different cytochrome P450 enzymes are involved in the oxidative processes, leading to the formation of various reactive metabolites (Stiborová et al. 2002, 2006).



Key Considerations for Health Effects Assessment

It is well established that genotoxicity and carcinogenicity are critical health effects for risk characterization of aromatic azo- and benzidine-based substances. However, when empirical data are available indicating that other health effects (e.g., effects on reproduction and development) may be of concern, these endpoints will also be considered in the assessment.

It has also been established that azo bond cleavage plays an important role in determining the health effects of many aromatic azo- and benzidine-based substances; therefore, the potential for cleavage for each substance is to be determined based on multiple lines of evidence including, 1) empirical data from the three types of studies described in the previous section (*in vivo* metabolism studies, *in vitro* metabolism studies, and mutagenicity testing under reductive conditions), 2) read-across among analogues and 3) (Q)SAR models. For insoluble/sparingly soluble dyes and pigments, evidence of bioavailability from repeated-dose studies can also be considered. In instances where there is sufficient evidence for cleavage, the genotoxicity and/or carcinogenicity of the resulting aromatic amine metabolites will be considered in the risk characterization.

Biological activity can also be attributed to the presence of one or more aromatic amines within the substance or through activation of the azo dyes via direct oxidation of the azo bond (i.e., Mechanisms II and III described above) (Brown and DeVito 1993). These two mechanisms are examined when empirical data or other evidence suggests that one of these pathways is utilized. Structurally related analogues may be used to inform the hazard potential of substances activated by these two mechanisms.

Many substances in this Substance Grouping have limited data. Accordingly, analogues will play an important role in characterizing metabolism of substances (e.g., potential for azo bond cleavage; activation through Mechanisms II and III described above). Analogues within the 358 substances in this Substance Grouping will be considered first, however, in the absence of

appropriate analogues within this Substance Grouping to inform the assessment, application of (Q)SAR may also be considered.

ECOLOGICAL ASSESSMENT CONSIDERATIONS

Ecological Exposure Assessment

The objective of the ecological exposure assessment is to characterize the nature, extent and magnitude of the exposure of ecological organisms to chemical substances in the environment. To this end, it is important to understand the uses, release patterns and environmental fate of substances. Concentrations in various environmental compartments (e.g., water, sediment, soil and air), can be derived and are referred to as predicted environmental concentrations (PECs). This information can be used to determine the level of risk posed by the substances to organisms living in or on each compartment. Exposure data discussed in this section are based on information from a variety of sources including publicly accessible sources and surveys issued prior to 2010 conducted under section 71 of CEPA 1999. A full analysis of data received from the recent Notice with respect to certain aromatic amines and aromatic azo- and benzidine-based substances (Canada 2011a) has not been completed at the time of the publication of this document; a more detailed analysis of exposure data, including data received from this most recent Notice, will be included in the draft screening assessment reports.

Uses and Release Patterns

The majority of substances in this Substance Grouping are used in industry as colourants. Since colorants are widely used in various applications such as textile, paper and leather dyeing, paint and coatings, inks, plastics, rubber and food (Hunger 2003; Herbst and Hunger 2004), many substances in this Substance Grouping can be used in these sectors. The production of these products is representative of several industry sectors, each of which consists of multiple facilities. Some of the substances in the Substance Grouping can also be found in non-colourant applications. There are also some industry sectors which use the products produced from one of the above sectors.

These substances may be released from industrial facilities, generally within wastewater. Generation of wastewater containing substances in this Substance Grouping can occur when water is used as a carrier for the substances in facility operations (e.g., paper dyeing) and disposed of at the end of the process. It can also occur when water is used in the cleaning of process equipment and then disposed, which is a common practice for many sectors. The wastewater generated from a facility may be treated on site; however, the degree of treatment may vary across sectors and from facility to facility. Many of the sectors involved with this Substance Grouping, including textile, leather, formulation and industrial use of coatings, rubber, food and pharmaceuticals, likely discharge their wastewater to local wastewater treatment systems. Another potential source of the release of colourants might be deinking plants which are a subset of some paper mills in Canada (Dyer 2001), using recycled paper as feedstocks and producing recycled paper products such as newsprint. Since certain substances in this Substance

Grouping are used in inks, they may be released from deinking plants to the aquatic environment.

In addition to the releases from industrial sources, consumer use of products such as textiles, paint, rubber, food and pharmaceuticals can also cause aquatic releases of some substances in this Substance Grouping via wastewater treatment systems. Examples, based on exposure assessments of various substances performed by Environment Canada, include (but are not limited to) paint residues that are washed down the drain, fine particles resulting from tire (rubber product) wear on city roads, which often end up in stormwater and colourants used in food and pharmaceuticals, which are found in sewage.

Mitigation by Wastewater Treatment

When a chemical enters a wastewater treatment system, it is subject to one or more of three removal mechanisms (Droste 1997; Stephenson and Blackburn 1998). The first mechanism is chemical transformation (e.g., biological degradation); the second mechanism is adsorption to non-aqueous media (sludge), such as solids and oil, followed by removal of the sludge from the wastewater; and the third mechanism is volatilization to air, which is promoted by aeration. Substances in this Substance Grouping are generally non-volatile and therefore are not expected to be removed by the volatilization mechanism or to be present in the air emissions from wastewater treatment systems. Most substances are expected to be removed mainly by the biodegradation and/or adsorption mechanisms. The removal efficiency varies depending upon the specific physical and chemical properties of the individual substances.

On-site wastewater treatment systems used by industrial facilities vary widely. For the sectors involved with this Substance Grouping, including container cleaning facilities, the types of treatment include solids settling, oil–water separation, filtration, and – at certain facilities – biological degradation. In addition to these types of treatment, the solids removed at paper mills can be further treated via digestion and dewatering to produce biosolids which can be, in particular, used for application on agricultural lands.

There are several different municipal wastewater treatment systems used in Canada (CWWA 2001). Primary mechanical systems use primary clarifiers (solids settling tanks) to remove solids. Coagulants and/or flocculants may be added to promote the settling and separation of solids from the liquid. The principal removal mechanism for these primary systems is adsorption to solids. Biodegradation is insignificant in these systems due to the combination of short residence times and limited chemical-eating bacteria.

Secondary mechanical systems consist of both primary and biological treatment. They are designed to biodegrade chemicals that cannot be removed by primary treatment alone. These systems therefore provide two removal mechanisms for non-volatile chemicals: adsorption to

solids and biodegradation. In Canada, the major portion (about 80%) of the municipal wastewater is treated by secondary systems, and activated sludge is the most common type used for biological treatment (CWWA 2001).

The solids removed initially from primary or secondary wastewater treatment systems are normally in the form of sludge. This sludge is further treated by digestion and dewatering. The resulting solids are commonly referred to as biosolids. A survey of the 50 largest sewage treatment systems in Canada which serve 48% of the Canadian population, revealed that the biosolids produced from these systems were disposed of mainly by incineration (48%) and land application (42%) (CG&S 2000). The remaining 10% of the biosolids were disposed of by other methods (e.g., landfilling and land reclamation). Soil exposure is therefore expected for substances in the azo group as a result of biosolids land application.

Lagoons are commonly used in Canada and are found in small municipalities. They may feature long residence times and seasonal discharges. Lagoons provide all three removal mechanisms (volatilization, adsorption and biodegradation), however the removal by these combined mechanisms has been found to vary from season to season (Wang et al. 2011). This variation is of particular importance to the exposure assessment, since certain industrial facilities are known to discharge their wastewater effluent to lagoons.

PEC Determination

In general, a key consideration in determining PECs in ecological exposure assessments is protection of the environment. When a substance is released to the environment, the area near and downstream of the release point often exhibits higher levels of exposure than other areas. Since this area is part of an ecosystem, it should be protected. Consequently, the levels of exposure in the area of release should be used as the basis for the PECs. For example, the estimated or measured concentrations of a substance near and downstream of the discharge point of a wastewater treatment system effluent should be selected for the PECs in the assessment of exposure of aquatic organisms to the substance.

Protection of the environment should also be considered in the event that exposure is subject to spatial or temporal variations. Exposure concentrations can have spatial variation when a substance is released at multiple geographical locations and temporal variation at specific locations if the substance in question is used only within a given period of time throughout the year (e.g., batch processing). The upper range of this spatial or temporal variation should be used to derive appropriate PECs so that the ecosystem is protected at all locations or at all times at each location.

Both lower and higher tiers of exposure assessment may be used in the PEC estimation process. Lower or higher tiers refer to lower or higher levels of detail in estimation calculations. In the lower tier, PEC estimates are made based on conservative assumptions in order to identify substances for which the exposure under consideration is likely of low concern. Although these

estimates do not necessarily reflect actual exposure, they provide an upper-bound estimate of exposure for screening purposes. When lower-tier results show a risk, these can be false positives and are therefore refined through higher-tier calculations. In higher-tier calculations, realistic data is used, when available, in place of conservative assumptions. In the absence of realistic data, stakeholders may be solicited to fill the data gap. The aim of this tiered approach is to focus resources on substances of higher potential concern.

Monitoring Data

Since azo- and benzidine-based substances are industrially synthesized and are not naturally occurring substances, any presence in the environment can be directly related to the release of the commercial substances at some part of their life cycle. Monitoring data for these substances in the Canadian environment are limited to historical concentrations in water and sediment. For example, Maguire and Tkacz (1991) investigated the occurrence of aromatic azo- and benzidine-based substances in the Yamaska River in southern Quebec in the mid- to late 1980s. Water, sediment and soil samples were collected from two sampling sites in two consecutive years to examine differences in dye concentrations. Sampling sites were located downstream from textile mills. River water collected in 1985 was found to have concentrations of Disperse Blue 79 (CAS RN 12239-34-8), which is contained in this Substance Grouping, ranging from 1.9 to 17.1 µg/L. By 1986, samples collected contained this dye at concentrations of 2.4–3.7 µg/L. River sediment contained the same dye at 0.1 mg/kg dry weight in 1985 and 4.2 mg/kg dry weight in 1986. Suspended solids collected from the Yamaska River contained Disperse Blue 79 at concentrations of 0.8 mg/kg dry weight in 1985 and 3.3 mg/kg dry weight in 1986.

Some additional environmental monitoring data, from outside of Canada, is available for azo- and benzidine-based substances in water, soil, sediment and sludge, but this is also limited. For instance, two azo acid dyes, Acid Orange 156 (CAS RN 68555-86-2) which is included in this Substance Grouping, and Acid Red 266 (CAS RN 57741-47-6) which is outside of the Substance Grouping, were found at concentrations in wastewater effluent of up to 48 and 12 µg/L, respectively, in the Coosa River Basin in Alabama in the United States in 1984 (Tincher 1986).

Given the lack of recent and relevant monitoring data, concentrations in the environment will be estimated based on available relevant information, including section 71 surveys, published literature, contract studies and release models.

Aquatic Exposure

Aquatic exposure results from the discharge of wastewater effluent into a receiving water body. If the wastewater generated from an industrial facility is sent to a local wastewater treatment system, the chemicals released from the facility are expected to be found in the receiving water. Since the area near and downstream of the discharge point of a wastewater treatment system is selected to capture the most vulnerable part of the aquatic environment, an appropriate dilution

of the effluent by the receiving water should be used in PEC determination. Normally, dilution up to a factor of 10, depending on the type and flow (in the case of rivers) of the receiving water, is considered appropriate at the immediate point of discharge.

Emission factors are an important component of PEC calculations. An emission factor is defined as a fraction of a substance's quantity released to a medium from an industrial facility or a specified use. Emission scenario documents (ESDs) published by the OECD are an important source of information, providing emission factors for many sectors and uses. These emission factors are, however, more generic than site specific emission factors and are often provided as a range, therefore there are uncertainties when emissions factors from ESDs are applied to specific facilities. There are also other sources of emission factors, such as information contained in various sector-specific technical documents from the US EPA and from industry-supplied data. For textile dyes, emission factors can be derived from a "fixation rate," which is the fraction of a substance's quantity which adheres to textile via dyeing operations. Since textile dyes are non-volatile substances, their emission factors for releases to wastewater can simply be calculated as one minus the fixation rate or unfixed fraction. Thus, a higher emission factor or a higher release to wastewater is expected for a dye with a lower fixation rate. As an example, Table 3 provides unfixed fractions for several common classes of textile dyes from different sources. These unfixed fractions are also applicable to azo- and benzidine-based dyes.

Table 7: Unfixed fractions of selected textile colourants from various sources

Dyestuff	**US EPA (1997)**	**OECD (2004)**	**ECHA (2003)**	**Danish EPA (Øllgaard et al. 1998)**	**ETAD (1995)**
Disperse dyes	5–25	1–12	1–12	0	1–12
Direct dyes	30	4–36	4–36	12	4–36
Reactive dyes	40	3–40	3–45	32	5–45
Acid dyes	20	2–15	2–15	10	2–15
Basic dyes	10	0–4	0–4	0–4	0–4
Pigment	—	—	0–2	2	—

Sediment Exposure

For certain substances, an equilibrium partitioning method may be used in determining sediment exposure. This method assumes that substances are adsorbed to sediment after their entry into the receiving water and reach an equilibrium between sediment and its overlying water. The sediment PEC is then simply a product of an equilibrium constant, commonly referred to as the sediment–water partition coefficient, and the aquatic PEC.

The magnitude of the sediment–water partition coefficient depends upon a substance's adsorption tendency and the sediment's adsorption capacity. For neutral organics, a substance's adsorption tendency is proportional to its octanol–water partition coefficient (K_{ow}), while the sediment's adsorption capacity is proportional to its organic carbon content, since the organic carbon is the principal phase for the adsorption. As a result, the sediment–water partition coefficient increases with a substance's K_{ow} or sediment's organic carbon content.

In estimating the sediment PEC for a given substance using the equilibrium method, the PEC in the overlying water will be determined as a sum resulting from both industrial and consumer or commercial releases. For example, a solvent dye used as a colourant in the formulation of printing ink is removed from recycled paper at a deinking plant. The dye is therefore contained in the wastewater generated from the plant. A portion of the dye is adsorbed to sludge when the wastewater is treated on site, while the remainder is released along with the effluent to the receiving water. Because of its hydrophobic nature, a large fraction of the dye in the receiving water partitions to the bed sediment. The PEC in the sediment may be derived from the PEC in the water column through the use of an appropriate sediment–water partition coefficient.

Given that most of the substances in this Substance Grouping are expected to exist as particles or charged substances, other techniques may be necessary to estimate exposure in sediment.

Soil Exposure

Soil exposure occurs as a result of land application of biosolids. The soil PEC can be estimated using the principle of mass balance. The boundary for the mass balance may be determined by the till or plough depth. The incoming mass is directly related to the biosolids application rate, while there are several mechanisms for the outgoing mass, including soil runoff, leaching into deeper soil layers, volatilization to air and degradation. The soil PEC is then determined by dividing the net mass by the soil volume within the boundary. A sufficiently long period of time should be selected in the mass balance calculations to ensure that the estimated exposure reflects the long-term nature of biosolids application.

Some substances in this Substance Grouping may be found in soil. For example, a solvent dye used in the formulation of printing inks for papers is removed at a deinking plant. A fraction of this dye in the plant's wastewater is then adsorbed to sludge during on-site wastewater treatment. The biosolids produced may be spread onto farmland. The resulting PEC in soil can be estimated from the concentration of the dye in biosolids and the maximum allowable application rate.

Given that most of the substances in this Substance Grouping are expected to exist as particles or charged substances, other techniques may be necessary to estimate exposure in soil.

Air Exposure

Air exposure is not expected to be important for the substances in this Substance Grouping, since, in general, they do not appear to have a significant potential to volatilize.

Environmental Fate

In this section, some general characteristics of the substances in this Substance Grouping 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. As research into these substances is still ongoing, this section will be primarily qualitative and high level in nature. More specific findings related to subgroups and individual chemicals will be presented in the assessment reports.

As shown above in Table 1, azo- and benzidine-based substances have a range of physical and chemical properties. Solubility in different media, partition coefficients and electrical charge are important parameters to consider in determining the fate of these substances in the environment. For operational purposes, it is possible to divide these chemicals into a set of ionic water-soluble dyes and a set of non-ionic sparingly water-soluble or insoluble colourants (pigments, disperse dyes and solvent dyes). The environmental fates of aromatic amines and simple benzidine transformation products are addressed separately.

Mass balance environmental fate models such as the Equilibrium Criterion model, otherwise known as EQC, are typically used to help characterize and quantify a chemical's behaviour in the environment (EQC 2003). However, EQC uses property-based relationships involving legacy chemicals (mostly non-ionic compounds). This model is generally not applicable for most azo- and benzidine-based substances, given that they likely exist as particles or aggregates (e.g., pigments) and ionizing chemicals (e.g., acid and basic dyes), which are outside of the domain of the model's applicability. While EQC may still be applicable for simple organic aromatic amines and benzidines, the environmental fate and compartmentalization of aromatic azo- and benzidine-based substances will be discussed qualitatively using information on physical-chemical properties.

Releases to Water and Sediment

If released to natural waters or wastewater in an untransformed state, ionic dyes (e.g., acid, direct and reactive dyes) are expected to have two predominant fates. Anionic (e.g., acid, direct and reactive dyes) and cationic (e.g., basic dyes) charged dyes will primarily bind to suspended organic matter due to electrostatic interactions and eventually settle out to bed sediments or wastewater sludge (ETAD 1995). Positively charged cationic dyes have an affinity for ionic substrates such as humic organic material, which has a net negative charge due to humic and

fulvic acids. Negatively charged ionic dyes have a high fixation rate with positively charged substrates and can adsorb to positively charged particulates (e.g., nitrogen-containing particles such as proteins and deoxyribonucleic acid [DNA]; Oster 1955). Some ionic dyes can also bind to organic material via hydrogen and van der Waals forces (Oster 1955).

Other factors, such as increasing molecular size, hardness of the water and salinity, as well as decreasing pH, are thought to favour some sorption of azo dyes to suspended solids (Øllgaard et al. 1998; HSDB 2012). It has been stated generally that, due to the recalcitrant nature of azo dyes in aerobic environments, they eventually end up in anaerobic sediments, shallow aquifers and groundwater (Razo-Flores et al. 1997).

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

Non-ionic colourants (e.g., pigments, solvent dyes and disperse dyes) are not expected to form electrostatic interactions with organic matter. However, the particulate character of pigments and some non-ionic dyes should have a key influence on their environmental fate.

The majority of organic pigments do not exist as individual molecules but are principally particles in the micrometre and submicrometre size range. The pigment powder is typically composed of primary particles (i.e., the crystal lattice of a pigment), aggregates and agglomerates. Manufacturers usually provide the physical specifications of their pigments, which include the average particle size of the pigment powder.

The density of azo-based pigments (Clariant 2007) and azo-based disperse dyes (Kojima and Ujima 1975) is typically greater than that of water; together with their low aqueous solubility, this suggests that they will preferentially partition by gravity to sediments or wastewater sludge. These dense particles may be transported short distances in water before settling occurs. Some solvent dyes also have densities greater than water and may act similarly.

Whereas pigments have very low experimental log K_{ow} and log dissociation constant (log K_D) values, disperse and solvent dyes may have some affinity for the organic components of suspended solids. It has also been shown that disperse dyes can bind to small particle size fractions of certain treated adsorbent minerals (e.g., calcined alunite; Özacar and Şengil 2002). With respect to wastewater treatment, most of the adsorption/desorption research on dyes in general has been done using activated sludge or carbon (ETAD 1995), with dyestuffs generally being adsorbed to the extent of 40–80% (Clarke and Anliker 1980). Flocculation or precipitation tends to occur with high calcium ion concentrations, which results in dyes settling out (Øllgaard et al. 1998).

While physical and chemical characteristics can provide important insight, the specific mechanism and adsorption potential of pigments and non-ionic dye structures are generally not

well understood. However, some studies (e.g., Yen et al. 1991) have shown that disperse dyes and their transformation products are found in sediment, so there is some evidence that settling to bed sediments takes place.

After partitioning to sediment or wastewater sludge, some azo colourants may bind reversibly and become resuspended, while others will bind irreversibly and remain buried. Certain azo colourants may also biotransform in sediment to aromatic amines. The fate of aromatic amines in sediment is discussed in further detail in the Environmental Persistence section.

Releases to Soil

There are two major routes for the release of azo colourants to soil: directly via the use or application of a colourant in the environment and indirectly via the application of wastewater sludge to agricultural land or deposition in landfills. While there is a paucity of scientific literature on this subject matter, in most cases, pigments, non-ionic dyes and ionic pigments released to soil are expected to stay in soil for reasons similar to those given for the preference of most azo colourants for sediment over water. Once in soil, biotransformation may occur.

While it has been noted that ionic dyes will have high to moderate mobility in soil due to low K_D values (Øllgaard et al. 1998), this is tempered with the finding that they may also undergo ion exchange processes with clay in soil, which would retard leaching (HSDB 2012). Specifically, acid dyes have an inherently high affinity for substrates, with fixation levels ranging from 85% to 98% for acid dyes with more than one sulfonic acid group (ETAD 1995).

Due to the insoluble and particulate nature of pigments and some non-ionic dyes, some of these will remain in soils if released to terrestrial environments. However, pigments and non-ionic dyes with low capacities to bind to organic matter are not likely to form strong chemical associations and may be washed out of soil.

Certain azo colourants may also biotransform in soil to aromatic amines. The fate of aromatic amines in soil is discussed in further detail in the Environmental Persistence section.

Releases to Air

A common characteristic of non-ionic dyes, ionic dyes and pigments is that they are not expected to be released to air and are not expected to partition to this compartment due to very low vapour pressures and Henry's Law constants (Øllgaard et al. 1998; HSDB 2012). Water-soluble dyes are intended for use in water-based treatments, which also limits their release. While pre-mixed colourants in their solid states may have some limited capacity for dispersal into the air as large particles, air is not considered to be a carrying medium for pigments and dyes, as these substances exhibit low or negligible volatility (Brown and Hamburger 1987; ETAD 1995; Øllgaard et al. 1998).

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

Environmental Persistence

The determination of the environmental persistence of azo colourants is complex and depends on a number of factors, including their intrinsic properties and the characteristics of the surrounding environment. Many colourants, especially pigments, are chemically designed to be durable in the environment and to maintain their colour in finished coatings, inks and paints over time (CPMA 2003). However, it is also commonly known that colours applied to many products tend to fade over time after exposure to sunlight and can separate and be released into water upon washing. This is one example of several phenomena that can result in the release and subsequent transformation of parent substances in the environment. This section will first discuss the main abiotic (e.g., photolysis or hydrolysis) and biotic (e.g., biodegradation) transformation and degradation processes that may apply to azo- and benzidine-based substances in the environment under aerobic and anaerobic conditions. The section will then explore the tools that are available to estimate the degradation rate (biodegradation and abiotic degradation) of the compound, as well as determine the identity of products resulting from transformation processes. Generally, when transformation products such as aromatic amines and benzidines are identified, they may be assessed along with the parent compound or in a separate assessment. The decision to assess a transformation product depends in part on the probability of obtaining the metabolite, on its stability and on its potential hazard to the environment (Environment Canada 2007b). In the case of the assessment of this Substance Grouping, key transformation products that fit hazard criteria with significant exposure potential (significant molar quantity) will be identified and addressed.

Abiotic Degradation and Transformation

Dyes are designed to possess a high degree of chemical and photolytic stability (Pagga and Brown 1986; Øllgaard et al. 1998); however, abiotic photodecomposition of some dyes under ultraviolet irradiation has been observed in the laboratory and in the environment (Shu et al. 1994; Shu and Huang 1995; Liakou et al. 1997a, b; Nansheng et al. 1997; Reutergådh and Iangphasuk 1997; Reddy and Kotaiah 2000; Harden et al. 2005). The rate of photodecomposition depends on oxygen levels, pH, light intensity and especially dye structure, as monoazo dyes have been found to be more easily decomposed than trisazo dyes (Hosono et al. 1993; Shu and Huang 1995; Liakou et al. 1997b; Nansheng et al. 1997; Reutergådh and Iangphasuk 1997). Photodecomposition rates of azo dyestuffs are generally slow in the natural environment (Øllgaard et al. 1998); however, photodecomposition may be accelerated in the presence of natural humic materials, probably through oxidation by single oxygen or oxy radicals (Brown and Anliker 1988).

Hydrolysis of reactive dyes has been observed, but its role in the degradation of azo substances is unclear (Øllgaard et al. 1998) and generally thought to be insignificant (Baughman and Perenich 1988).

Numerous chemical degradation and transformation processes of azo substances and colourants have been studied (Anjaneyulu et al. 2005; Shirin and Balakrishnan 2011). Oxidation using powerful oxidizing agents such as chlorine, ozone or Fenton's reagents is the most commonly used chemical degradation process for the treatment of industrial wastewater containing various types of colourants (Anjaneyulu et al. 2005). Several aromatic azo substances and other types of colourants are susceptible to zero valent iron (Fe^0) reductive transformation to produce aromatic amines (Larson and Weber 1994; Weber 1996; Feng et al. 2000; Shirin and Balakrishnan 2011). While these studies have been undertaken in severe chemical conditions, they may provide insight into the type of environmental or biological transformations expected for some of the azo- and benzidine-based substances as well as the identity of their potential transformation products.

Biodegradation and Transformation

Aerobic Processes

According to the Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers, dyes are, with some exceptions, considered essentially non-biodegradable under aerobic conditions (ETAD 1995). Repeated evaluation of ready and inherent biodegradability using accepted screening tests (e.g., OECD Guidelines for Testing Chemicals) have been consistent with this assumption (Pagga and Brown 1986; ETAD 1992). However, recent research indicates that some degree of aerobic degradation of azo substances by bacteria, fungi and yeasts may be achieved under specific conditions.

Bacteria

While rare, aerobic biodegradation of azo substances has been observed under experimental conditions with specific bacterial cultures (Kulla 1981; Zhang et al. 1995; Blumel et al. 1998; Sarayu and Sandhya 2010). The degrading ability of the bacterial strain, however, is usually restricted to a specific simple dye structure (Kulla 1981; Zhang et al. 1995; Erkurt et al. 2010) following the adaptation of the bacteria via long-term aerobic growth in the presence of the substance (Stolz 2001; Sandhya 2010). The mechanism of degradation involves the synthesis of an azoreductase able to reductively cleave the azo group in the presence of oxygen (Stolz 2001; Sandhya 2010). Under aerobic conditions, monooxygenase and dioxygenase enzymes may catalyze the incorporation of oxygen into the aromatic ring of organic compounds prior to ring fission (Sarayu and Sandhya 2010; Saratale et al. 2011).

Fungi

Filamentous fungi are ubiquitous and are usually found in soil, living plants and organic waste material (Saratale et al. 2011). Many experimental studies have shown the ability of different types of filamentous fungi to degrade a wide variety of colourants in test systems under aerobic conditions, as summarized by Erkurt et al. (2010). Fungi have also been shown to decolourize pigments (Banat et al. 1996). Most studies on fungal azo biodegradation have focused on developing processes for the complete mineralization of the compounds (Machado et al. 2006). The ability of fungi to degrade a large range of organic chemicals, including colourants, results from the relatively non-specific nature of their lignin degrading (ligninolytic) enzymes (Christian et al. 2005). The main enzymes involved in the degradation of dyes (Chagas and Durrant 2001; Bor et al. 2004; Eichlerova et al. 2005; Unyayar et al. 2005; Erkurt et al. 2007; Murugesan et al. 2007) and pigments (Banat et al. 1996) are three oxidative enzymes—namely, manganese peroxidase, lignin peroxidase and laccase. Lignin peroxidase and manganese peroxidase are oxidoreductases, while laccase is a phenoloxidase (Erkurt et al. 2010). Synthesis and secretion of these enzymes in fungi are often induced by limited levels of carbon or nitrogen nutrients (Wesenberg et al. 2003; Erkurt et al. 2010). A general mechanism of ligninolytic enzyme azo dye oxidation is the formation of a carbonium ion, followed by nucleophilic water attack, forming benzoquinone and a diazen derivative (Dias et al. 2010). The degradation rate of dyes has been observed to decrease with high nitrogen concentrations (Øllgaard et al. 1998); however, not all fungi have their ligninolytic systems regulated by nitrogen concentration (Machado et al. 2006), indicating that these enzymes are able to degrade a variety of azo substances.

Yeasts

Similarly to fungi, several studies have shown the ability of yeasts (e.g., *Candida* species) to enzymatically degrade azo dyes under aerobic conditions, as summarized by Dias et al. (2010). The oxidative cleavage of azo dyes involves the ligninolytic enzymes manganese peroxidase, lignin peroxidase and laccases described for fungi and is also relatively non-specific (Dias et al. 2010).

Anaerobic Processes

Upon their release to sediment, disperse dyes and pigments are expected to eventually settle to the aerobic layers of surface sediment, where they will persist until sediment burial creates conditions suitable for reducing conditions due to the absence of oxygen. Therefore, the persistence of these substances under anaerobic conditions constitutes an important aspect of their ecological risk assessment.

Under anaerobic and anoxic conditions, many azo substances are vulnerable to bacteria-mediated cleavage of their azo bond (Brown and Laboureur 1983; Baughman and Weber 1994; Weber and Adams 1995). Some azo disperse dyes may degrade completely in sediment at depths where anoxic conditions persist (Razo-Flores et al. 1997). However, complete degradation of the aromatic azo- and benzidine-based substances may not always occur, since metabolites resulting

from azo bond cleavage may persist under anaerobic conditions (Pinheiro et al. 2004). Also, the environmental persistence of pigments in anoxic environments is an area of some uncertainty. For example, while β-naphthol pigments also have azo chromophores in their structure, a possible transformation potential of these pigments in the absence of oxygen has not been found in the literature. In principle, the crystal would have to dissolve first, releasing its constituent molecules. The azo bonds in these molecules would then be available for reduction.

Bacteria

Bacteria-mediated azo bond cleavage via azoreductase enzymes is non-specific with regard to the organisms involved and the substances reduced (Anjaneyulu et al. 2005). The cleavage of the azo bond occurs in two stages in the environment, as presented in Figure 7, where two electrons are transferred at each stage to the azo substance, which acts as a final electron acceptor (Guo et al. 2010).

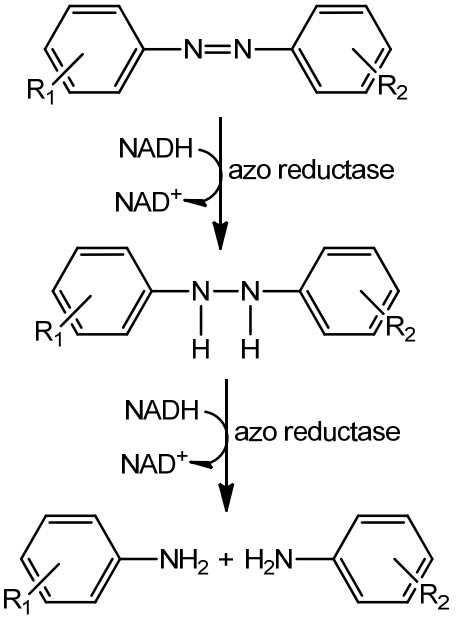


Figure 7: Azoreductase mechanism in the environment (Guo et al. 2010).

Most dyes are xenobiotic; many are polar, and most are large molecules for which there may not be carrier proteins. These molecules are unlikely to enter the interior of the cell, where they could be utilized by non-specific reductase enzymes (Khalid et al. 2010). While the mechanism of azo dye reduction is not completely known (Hong et al. 2007), it is presumably mainly an extracellular process (Khalid et al. 2010) involving redox mediators shuttling electrons from bacteria to the azo substances (Keck et al. 1997; Rau et al. 2002; Rau and Stolz 2003). Three main mechanisms have been presented to explain azo reduction: direct enzymatic reduction with azoreductase, indirect/mediated reduction involving azoreductase and, finally, chemical reduction with reductants such as sulfides (Guo et al. 2010).

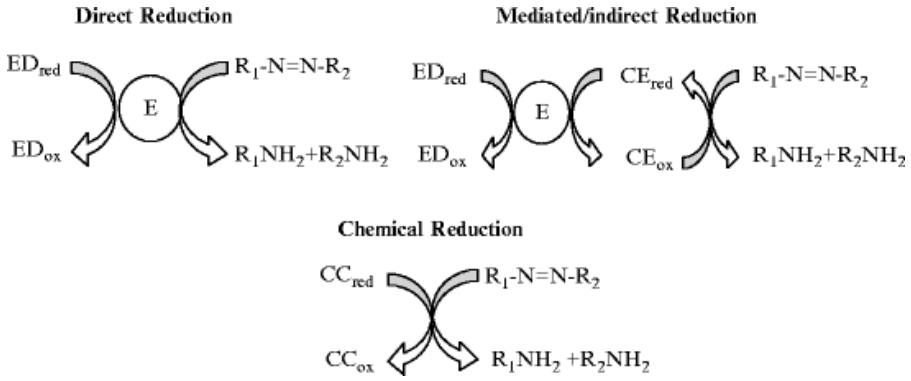


Figure 8: Mechanisms of azo dye bioreduction (Guo et al. 2010).

Direct enzymatic reduction indicates that chemical reduction of the azo colourants would be conducted by specific enzymes catalyzing only the reduction of azo dyes; however, such specific enzymes have not been identified for anaerobic bacteria in the environment (Guo et al. 2010). It is generally understood that mediated/indirect reduction involving a variety of redox mediators is the main mechanism of dye reduction (Guo et al. 2010). Such reduction likely involves many bacterial strains possessing non-specific cytoplasmic enzymes acting as azoreductases and participating in the transfer of electrons to the azo dye (Walker 1970; Russ et al. 2000). For example, a flavoprotein is a purified enzyme from one azo-degrading bacterial strain and is capable of catalyzing the reduction of nitroaromatics (Van der Zee 2002). Some flavin-based compounds and quinine-based compounds have been extensively reported as redox mediators (Dos Santos et al. 2004). Examples of the former include flavin adenide dinucleotide and FMN and of the latter include anthraquinone-2,6-disulfonate, anthraquinone-2-sulfonate, riboflavin (vitamin B_2), cyanobalamin (vitamin B_{12}) and lawsone (2-hydroxy-1,4-naphthoquinone).

Finally, the reduction of the azo dye may be catalyzed extracellularly by the action of mediator compounds formed during the metabolism of certain substrates by bacteria (Russ et al. 2000; Guo et al. 2010). These types of mediators, such as sulfides generated by sulfate-reducing bacteria, may participate in the reduction of azo substances through chemical reduction (Guo et al. 2010).

Factors Affecting Transformation and Degradation

While anaerobic azo reduction is a fairly non-specific process, the rate of transformation has been observed to vary depending on the chemical structure and the presence of some specific functional groups (Yen et al. 1991). Dyes with simple structures and low molecular weights exhibit higher rates of colour removal in wastewater treatment systems compared with high molecular weight dyes, with substitution of electron withdrawing groups (e.g., SO_3H or SO_2NH_2) in the *para* position of the phenyl ring relative to the azo bond (Sani and Banerjee 1999; Pearce

et al. 2003; Hsueh et al. 2009; Saratale et al. 2011). Azo substances with hydroxyl or amino groups are more likely to be transformed than those with methyl, methoxy, sulfo or nitro groups (Saratale et al. 2011).

Transformation Products and their Fate and Persistence

As indicated in previous sections, the transformation of azo substances under anaerobic conditions may release aromatic amines (Yen et al. 1991; Pinheiro et al. 2004). The exposure of aquatic organisms to these biotransformation products in anoxic sediments will depend on their fate and persistence in the environment.

Aromatic amines are generally resistant to biodegradation under anaerobic conditions (Brown and Hamburger 1987). Naphthalenamines have shown recalcitrance under anoxic denitrifying, sulfate-reducing and methanogenic conditions in flooded soils (Al-Bashir et al. 1994). Aniline is extremely recalcitrant to degradation under methanogenic conditions (De et al. 1994). However, biodegradation of some compounds has been observed to occur after acclimatization of sludge in wastewater treatment test systems (O'Connor and Young 1989; Ekici et al. 2001) and in sediments under anaerobic conditions. Bacterial mediated dehalogenation of dichlorobenzidine to benzidine has been observed in anaerobic sediments (Nyman et al. 1997). This progressive dehalogenation was expected to yield a greater total concentration of aromatic amines in the solution phase and greater potential for transport in the environment (Nyman et al. 1997). The mineralization of aromatic amines under anaerobic conditions is dependent on their bioavailability, which is limited by sorption and desorption (Al-Bashir et al. 1994).

The biodegradation of aromatic amines under aerobic conditions varies (Baird et al. 1977). The biodegradation potential of aromatic amines in aerobic sediment is dependent on their chemical structure (Bornick et al. 2001). Their biodegradation potential ranges from non-biodegradable to very high, depending on the type, number and position of the substituents in the benzene ring (Alexander and Lustignan 1966; Pfarl et al. 1990; Okey and Stensel 1996; Bornick et al. 2001). Aerobic degradation of the aromatic amines can occur through bacterial fission of the aromatic ring structure (Gottlieb et al. 2003).

Fate

Sorption and desorption are two important fate processes for aromatic amines in aquatic, sediment and soil systems (Chen and Nyman 2009), which govern their transformation and transport (Colon et al. 2002). Sorption of aromatic amines to sediment involves an initial rapid, reversible sorption step followed by a slower, irreversible step (Colon et al. 2002). Sorption involves a series of main mechanisms (Colon et al. 2002; Chen and Nyman 2009), including electrostatic interaction (i.e., cation exchange process), hydrophobic partitioning, labile bonding (i.e., hydrogen bonding, van der Waals forces, dipole–dipole interactions) (Spurlock and Biggar 1994; Huang et al. 1997; Chiou et al. 1998; Xia and Ball 1999) and covalent bonding.

While covalent bonding may occur at the first rapid sorption step, it generally occurs as the second slower step (Weber et al. 2001; Colon et al. 2002). It may involve nucleophilic addition of the amino functional group to electrophilic sites or oxidative mechanisms resulting in the formation of radical species that couple with sediment-bound radicals (Spurlock and Biggar 1994; Huang et al. 1997; Chiou et al. 1998; Xia and Ball 1999; Weber et al. 2001; Colon et al. 2002). It is noted that sorption of *ortho*-substituted aromatic amines is significantly less than that observed for the *meta*- and *para*-substituted anilines (Colon et al. 2002). Some of the sorption mechanisms are pH dependent. A shift of sorption mechanisms from cation exchange to hydrophobic partitioning, covalent binding or both has been observed for benzidine when the pH increased from 3 to 7 (Chen and Nyman 2009).

Modelling Biodegradation

A variety of models can be used to predict biodegradation. All modelling of biodegradation uses the SMILES code to compare the structural attributes of a compound with those of the chemicals in the training set. Some models, such as CATALOGIC (©2004–2012), are kinetic and report quantitative results, while others are additive fragment-based models, such as BIOWIN (2010) probability models and TOPKAT (2004), or offer qualitative results (BIOWIN Survey Models 3 and 4). The BIOWIN 4 Primary Survey Model can be considered a primary biodegradation model (some transformation of parent structure). The BIOWIN Japanese Ministry of International Trade and Industry (MITI) linear and non-linear probability models, CATABOL (©2004–2012), TOPKAT and BIOWIN 3 (ultimate survey model) are ultimate degradation models (complete mineralization).

None of the models mentioned above are specifically designed to give a determination of "persistence" in terms of half-lives. Rather, they are designed to give an evaluation of whether a substance is likely to biodegrade quickly or slowly or be more persistent than not. The half-lives can be extrapolated from these results, assuming first-order rate kinetics or using a read-across approach. These results are interpreted in order to make a conclusion on persistence based on the definition under the CEPA 1999 *Persistence and Bioaccumulation Regulations* (Canada 2000).

The formation of degradation products from organic compounds may also be systematically assessed with CATALOGIC (©2004–2012). CATALOGIC, which is the kinetic version of CATABOL (©2004–2012), is a mechanistic modelling approach developed to quantitatively assess the biodegradability of chemicals and their biodegradation pathways (Jaworska et al. 2002). CATALOGIC and CATABOL predict the most probable biodegradation pathway, the distribution of stable metabolites and the "ready biodegradability" endpoints of a chemical, such as cumulative theoretical oxygen demand (MITI-I) and extent of carbon dioxide production (OECD 301B) (Dimitrov et al. 2004). Interesting features associated with these models are that they estimate the biodegradability of a chemical based on the entire biotransformation pathway instead of the parent structure alone and consider the effects of adjacent fragments of the chemical before executing each transformation step (Pavan and Worth 2006).

The core of CATABOL (©2004–2012) is the biodegradability simulator, including a library of more than 1000 hierarchically ordered individual transformations (catabolic steps) and a matching substructure engine providing their subsequent performance (Pavan and Worth 2006). The set of transformations identified is divided into two types: "spontaneous" and "catabolic." "Spontaneous" transformations may be biotic or abiotic, including, for example, spontaneous hydrolysis. "Catabolic" transformations describe only biotic processes (Pavan and Worth 2006). The CATABOL pathway library includes an azo bond reduction degradation pathway. Indeed, Blumel et al. (1998) observed the aerobic catabolic breakdown of a 4-carboxy-4′-sulfoazobenzene-utilizing bacterial strain (S5) adapted from *Hydrogenophaga palleroni* S1, contrary to the general knowledge of anaerobic azo compound reduction. While aerobic azo bond reduction is fairly rare, CATABOL results will be used to identify possible degradation products specific to each azo compound. Key degradation products will also be addressed in other sections of the assessment (e.g., bioaccumulation, effects and exposure).

Bioaccumulation

Bioaccumulation is the tendency of a substance to be absorbed from an environmental medium (e.g., water, soil) or prey item into the tissues of an organism, where it may accumulate (Environment Canada 2007b). In order to be absorbed, the chemical must first be in a bioavailable form. After a chemical is absorbed or ingested by an organism, it is important to determine whether the body burden of the chemical resulting from bioaccumulation can reach internal levels that cause adverse effects in the organism. In general, a substance that is metabolized or broken down readily by an organism will have less potential to cause adverse effects than a substance that cannot be readily eliminated from the tissues of an organism (assuming that the breakdown products of the substance are non-toxic). This is because the internal concentration of a non-metabolized substance in an organism is higher than that of a metabolized substance, and so the tissue concentration will build up slowly over time, creating a higher potential for internal damage to the organism.

Experimental data for traditional bioaccumulation metrics exist, but they are minimal and mostly restricted to the water compartment for this Substance Grouping. Whenever possible, data from traditional bioaccumulation tests will be used as read-across for other substances in order to maximize their information value. A limited number of experimental log K_{ow} values, bioconcentration factors (BCFs) and bioaccumulation factors (BAFs) are available for the substances in this Substance Grouping. In particular, data on bioaccumulation in soil and sediment are minimal and limited to a few high-volume substances. Also, it is difficult to model these parameters for the majority of the substances in this Substance Grouping, since they are outside the various model domains of applicability.

Another difficulty with respect to using traditional bioaccumulation metrics for this Substance Grouping is the unreliability of some of these data. For instance, experimental log K_{ow} values for pigments and some sparingly water-soluble dyes are often not reliable, as the substances are so

insoluble in both media that the test is very difficult to perform. Since modelled log K_{ow} values are also unreliable, the ratio log (C_o/C_w) is often used instead, where solubility in octanol (C_o) and solubility in water (C_w) are measured separately and compared. This approach is supported by the observation that partitioning into octanol is an indicator of the potential of a substance to partition into the lipid phase of aquatic biota (Bertelsen et al. 1998) and, for pigments, the observation that reduced solubility in octanol translates into a similarly reduced BCF and BAF in an aquatic organism (Banerjee and Baughman 1991).

While bioaccumulation data for substances in this Substance Grouping are somewhat scarce, general trends have emerged over the years. For example, data from Anliker et al. (1981) show that the very water soluble ionic dyes tend to have low log BCF values of approximately −1 to 1. The authors suggested that this low bioaccumulation potential is a result of these dyes adhering to the outside of the fish or to the intestine. Also, their absolute fat solubility is low (Brown and Hamburger 1987). Studies by ETAD (1991) demonstrated that the log K_{ow} values of certain reactive dyes were very low (below zero) and that the dyes did not show any tendency to bioaccumulate in flow-through tests using carp.

It is noted that even though non-ionic disperse dyes may have higher log K_{ow} values (e.g., > 3), they do not seem to exhibit the capacity to bioaccumulate to a great extent. Anliker and Moser (1987) hypothesized that this may be due to the pronounced aggregation tendency of disperse dyes, which makes transport across membranes difficult. The results of fish bioconcentration studies with disperse dyes by Anliker (1986) and Anliker et al. (1988) have been consistent with this hypothesis.

Bioaccumulation of pigments in fish is also not typically observed (e.g., Anliker et al. 1981, 1988). This may be because of their low solubility in octanol, large particle size and large cross-sectional diameter, which make membrane permeation very difficult.

Where relevant, some metabolic data from small mammalian species (e.g., rat and mouse models) used in the human health effects assessment may be considered as a surrogate for wildlife data to inform bioaccumulation of mammals in the terrestrial environment in Canada.

Both measured and estimated log BCFs for the potential aromatic amine degradation products of aromatic azo-based colourants have been found to be generally quite low (HSDB 2012), in the range of 1.5–2.0.

Additional Parameters Influencing Bioaccumulation

Molecular size, as described by cross-sectional diameter, and bioavailability, as described by physical state, are considered to be important mitigating parameters for the determination of the bioaccumulation potential of substances in this Substance Grouping.

It has been hypothesized that high molecular weight and cross-sectional diameter make it difficult for azo colourants such as disperse dyes and pigments to cross biological membranes (Anliker et al. 1988). Investigations relating to fish BCF data and molecular size parameters (Dimitrov et al. 2002, 2005) suggest that the probability of a molecule crossing cell membranes as a result of passive diffusion declines significantly with increasing maximum diameter (D_{max}); the probability of passive diffusion decreases appreciably when the maximum diameter is greater than about 1.5 nm and much 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}) above 1.1 nm.

Arnot et al. (2010) pointed out that there are no clear relationships for establishing strict molecular size cutoffs for assessing bioaccumulation potential. However, the report does not dispute the notion that a reduction in uptake rate can be associated with increasing cross-sectional diameter, as demonstrated by Dimitrov et al. (2002, 2005). Many of the substances in this Substance Grouping have high molecular weights, and the maximum diameter of the majority (approximately 75%) of the 358 substances is greater than 1.5 nm, suggesting that a potential exists for a significantly reduced rate of uptake from water via the gills of fish.

Arnot et al. (2010) emphasized that there are uncertainties associated with the thresholds proposed by Dimitrov et al. (2002, 2005) and Sakuratani et al. (2008), since the BCF studies used to derive them were not critically evaluated. Arnot et al. (2010) 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 in” equals “slow out”). Thus, significant bioaccumulation potential may remain for substances that are subject to slow absorption processes if they are also slowly biotransformed or slowly eliminated by other processes, such as fecal egestion. Consequently, when evaluating bioaccumulation potential, molecular size information is considered with care and used together with other relevant lines of evidence in a weight of evidence approach.

Characteristics such as melting or decomposition point, specific physical state at standard temperature (i.e., liquid, gas, solid) and particle size can be used in the weight of evidence for bioaccumulation potential.

Melting or decomposition point has been commonly used to help explain the environmental fate of colourants (e.g., Anliker and Moser 1987) and is an important parameter, since it affects solubility, which in turn influences bioavailability and the transport of a substance to active sites within an organism (Øllgaard et al. 1998). Melting or decomposition point tends to increase with the size of a molecule, since the molecular surface area available for contact with other molecules increases (Dearden 1991). Substances that have a high degree of chemical stability

with a high melting point tend to be less bioavailable in water. For many colourants (pigments, certain dyes and certain aromatic amines), the term “decomposition point” is used instead of “melting point,” as they are known to chemically break down at high temperatures (e.g., > 200°C) rather than melt or liquify (ETAD 1995). Many substances in this Substance Grouping have high decomposition points, demonstrating high chemical stability.

In addition, many substances in this Substance Grouping are found in a solid state for applications such as colouring of coatings or plastics. In these cases, especially for non-ionic substances, there is a decreased likelihood of aqueous bioavailability, as the substance may be relatively inert. In other cases, when the substances are in mixtures in a liquid state, the potential for bioavailability is considered higher.

Some colourants, such as organic pigments, typically have a certain proportion of their particle size spectra in the nanoparticle range (NPIRI 2000). Nanoscale materials are informally defined as substances having at least one dimension less than 100 nm. There is increasing evidence to the effect that nanoparticles can be absorbed by non-specific biouptake pathways, such as pinocytosis (Leroueil et al. 2007). At present, the bioaccumulation mechanisms and potential of these particles are poorly understood, as is the nature of the relationship between their bioaccumulation and their toxicity. Furthermore, certain less commonly considered environmental fate processes may have an important influence on the propensity of the pigment nanoparticles to be taken up by biota (e.g., importance of aggregation in nature; Wiesner et al. 2006). However, as little is known related to the environmental fate processes of nano pigments, the relative contribution of nano size fractions to any hazardous effects from traditional toxicity tests is an area of uncertainty.

Ecological Effects

It is important to characterize the type and magnitude of adverse ecological effects, direct or indirect, that could occur following exposure to a substance (or a degradation product of the substance) in the environment. In order to understand the effects of a particular class or subgroup of substances, it is necessary to consider their particular modes of action. Often, chemically similar substances (e.g., with the same major functional groups) are expected to exhibit the same mode of action related to effects on organisms. While the substances in this Substance Grouping share certain structural features and uses, there is still a high degree of chemical diversity, which makes it likely that several modes of action may exist (e.g., because of different substituent groups). It has been posited that at least three distinct toxic modes of action, “non-polar narcosis,” “polar narcosis” and “unknown reactive,” should be considered when predicting the toxicity of these substances. Polar narcosis is the most severe and would be the most conservative assumption. Additionally, more reactive substances may exhibit some degree of protein and DNA binding, which should be investigated. Also, while a considerable number of experimental toxicity endpoints have been investigated over many years, there is still a great deal

of uncertainty, as some effects may be demonstrated, but the specific mechanisms of toxicity are often unknown.

Pigments, non-ionic dyes and ionic dyes have been shown to exhibit a variety of lethal and sublethal effects in different media. Acute effects occur at concentrations that vary from less than 1 mg/L to well over 1000 mg/L (Øllgaard et al. 1998). Overwhelmingly, ecological effects data have been focused on the aquatic environment, while soil and sediment toxicity testing of these substances has been largely ignored.

It is very difficult to analytically measure the soluble concentrations (dissolved phase) to which the organisms are exposed. As a result, toxicity values are often not accurate, since the reported endpoint value is actually at a nominal concentration (sometimes orders of magnitude higher than the true measured concentration) or at the solubility limit. In these instances, the solubility limit may be taken as the effect concentration, as a conservative measure. However, even if certain aromatic azo- and benzidine-based substances are not soluble, they may be ingested and can either metabolize within the organism, thus detoxifying the chemical, or degrade to an equally or potentially more harmful substance, such as aromatic amines (including benzidines).

In the ecological effects section of the assessments, key potentially harmful degradation products will be considered. Standard acute and chronic effects in aquatic and terrestrial media will be outlined, as described below.

Effects in the Aquatic Environment

Since many substances in this Substance Grouping are water soluble and/or may end up in bed sediments, it is important to consider effects in the aquatic compartment, although limited aquatic toxicity tests are available. The majority of these tests are acute and focus on common aquatic laboratory organisms, especially invertebrates and fish. Invertebrates tested include *Daphnia magna*, *Ceriodaphnia* and *Daphnia pulex* (water fleas) and *Hyalella azteca* (scud). Tests on fish focus on the common *Oncorhynchus mykiss* (rainbow trout), *Oryzias latipes* (Japanese medaka), *Pimephales promelas* (fathead minnow), *Cyprinus carpio* (common carp) and *Brachydanio rerio* (zebrafish) species. Some studies are also available on algae (e.g., *Selenastrum capricornutum*, *Chlorella vulgaris* and *Scenedesmus subspicatus*), as well as certain bacteria. A few tests are also available on amphibians (e.g., *Silurana tropicalis* and *Pleurodeles waltl*).

Recently, some sediment-based endpoints for certain yellow and red pigments (Pigment Yellow: 12, 13, 83 and 176; Pigment Red: 48-2 and 112) as well as a reactive dye (Reactive Black 5) have been made available to the European Commission's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Sediment-dwelling organisms that were tested include *Lumbriculus variegatus*. While sediment toxicity data are still very limited for many substances in this Substance Grouping (pending reliability analysis of the studies), these new endpoints may be used as read-across for several chemicals.

Apart from biotic effects on organisms, some substances in this Substance Grouping may also cause some abiotic or physical effects. While this is still an area of uncertainty, the principal abiotic effect that has been discussed in the scientific literature relates to the physical effect of light inhibition or shading of algae in aquatic media. Algae appear to be among the most sensitive organisms to all dye classes. For example, when algae were exposed to dye concentrations of 1 and 10 mg/L for 7 and 14 days, it was found that 15 dyes (27% of the dyes tested) strongly inhibited growth at the test concentration of 1 mg/L after 7 days of incubation (Brown and Anliker 1988).

However, these results should be considered carefully, since there is some evidence that algal colour shading tests are flawed and that no significant differences exist between colours and non-colours, because the method is unsatisfactory (Cleuvers and Weyers 2003). It is posited that when the test flasks are shaken, algae are exposed to some level of light, which leads to a higher growth rate than expected in comparison with no exposure to light. This can result in a reduced shading effect and consequently an underestimation of chemical toxicity.

Effects in the Terrestrial Environment

Very few ecologically relevant studies on terrestrial effects exist. However, some soil endpoints have been submitted to REACH for certain pigments and dyes. For example, data from studies on soil-dwelling earthworms (*Eisenia fetida*/*Eisenia andrei*) will be evaluated and considered, when relevant, in relation to potential ecological exposure to wastewater treatment plant sludge applied to land.

Other Ecological Effects

Effect endpoints of primary importance in ecological risk assessments under CEPA 1999 have typically been direct effect measures (e.g., effect concentrations for 20% of the organisms [EC_{20}s] or median lethal concentrations [LC_{50}s]) indicating impaired survival, growth and/or reproduction. In some cases, consideration for carcinogenicity data has also been used where an established link between environmental exposure to a substance and incidence of cancer in certain wildlife species has been observed. Acknowledging that cancer generally occurs infrequently in wild animals and that it is difficult to assess the potential for the manifestation of cancer endpoints in individual organisms and to estimate the overall impact on individuals or local populations of organisms, information on cancer potency could nevertheless be more systematically integrated into the evaluation of ecotoxicity as one line of evidence for adverse effects. When there is clear evidence that a substance causes cancer in laboratory animals (particularly through a genotoxic mechanism), such information could be considered to contribute to the weight of evidence suggesting potential to cause ecological harm under CEPA 1999—as was done in the Priority Substances List (PSL) assessment of dioxins and furans (Canada 1990). The information could be either experimental or derived from molecular modelling software suggesting structural alerts for genotoxicity or mutagenicity.

Critical Toxicity Value

A critical toxicity value (CTV) is generally the lowest concentration of a substance, from the acceptable available data, at which an adverse effect was observed in the most sensitive species. The selection of toxicological endpoints is aimed at maintaining the survival and reproduction of populations that are expected to be exposed to a substance (Environment Canada 2007b). When selecting a CTV, long-term (chronic) toxicity data are generally preferred over short-term (acute) data, since environmental exposure can be continuous over the long term and since there there is a higher chance that equilibrium can be reached, especially for substances with low solubilities. Acceptable measured data and predicted data may both be considered in a weight of evidence approach (Environment Canada 2007b).

In the case of this Substance Grouping, it is not yet possible to identify the most sensitive endpoints for each subgroup (representative CTVs) or individual substance. Key endpoints will be evaluated for reliability based on standard criteria in the form of Robust Study Summaries and discussed in the assessments.

Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) is derived from the CTV and represents the concentration of a substance in the environment that is not expected to induce any adverse effects in a population typically following chronic or long-term exposure (Environment Canada 2007b). PNECs are calculated by dividing the CTV by an appropriate assessment factor. The size of the assessment factor applied is reflective of the uncertainty in the available data and the level of extrapolation needed. In practice, assessment factors vary in magnitude and are used to account for such factors as extrapolation from acute to chronic effects, extrapolation from single-species laboratory tests to ecosystem impacts and variations in sensitivity between species or between individuals within a species.

PATH FORWARD

Industry and other interested stakeholders are invited to submit comments during a 60-day public comment period on the content of this Draft Technical Background Document to the Substances Management Information Line. Comments should be submitted by September 12, 2012, since comments received will be considered in the development of the Final Technical Background Document, anticipated for release in winter 2012/2013. Comments on the Draft Technical Background Document should be submitted to the address provided below:

Substances Management Information Line
Chemicals Management Plan
Gatineau, QC
K1A 0H3

Telephone: 1-800-567-1999 (in Canada) or 819-953-7156
Fax: 819-953-7155
E-mail: substances@ec.gc.ca

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APPENDIX I: List of Substances in the Aromatic Azo- and Benzidine-Based Substances Group

Table I-1: Benzidine-Based Pigments

CAS RN	DSL name (C.I. name)
5102-83-0	Butanamide, 2,2′-[(3,3′-dichloro[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[*N*-(2,4-dimethylphenyl)-3-oxo- (Pigment Yellow 13)
5567-15-7	Butanamide, 2,2′-[(3,3′-dichloro[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[*N*-(4-chloro-2,5-dimethoxyphenyl)-3-oxo- (Pigment Yellow 83)
6358-85-6	Butanamide, 2,2′-[(3,3′-dichloro[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[3-oxo-*N*-phenyl- (Pigment Yellow 12)
7147-42-4	Butanamide, 2,2′-[(3,3’-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[*N*-(2-methylphenyl)-3-oxo-
29398-96-7	[1,1’-Biphenyl]-4,4′-diamine, *N,N*′-bis(2,4-dinitrophenyl)-3,3′-dimethoxy- (Pigment Brown 22)
78952-70-2	Butanamide, 2-[[3,3′-dichloro-4′-[[1-[[(2-chlorophenyl)amino]carbonyl]-2-oxopropyl]azo][1,1′-biphenyl]-4-yl]azo]-*N*-(2,4-dimethylphenyl)-3-oxo-
90268-24-9	Pigment Yellow 176 (Pigment Yellow 176)

Table I-2: Benzidine-Based Dyes

CAS RN	DSL name (C.I. name)
72-57-1	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethyl[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-, tetrasodium salt (Direct Blue 14)
91-92-9	2-Naphthalenecarboxamide, *N,N*′-(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis[3-hydroxy- (Azoic Coupling Component 3)
573-58-0	1-Naphthalenesulfonic acid, 3,3′-[[1,1′-biphenyl]-4,4′-diylbis(azo)]bis[4-amino-, disodium salt (Direct Red 28)
992-59-6	1-Naphthalenesulfonic acid, 3,3′-[(3,3′-dimethyl[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[4-amino-, disodium salt (Direct Red 2)
1937-37-7	2,7-Naphthalenedisulfonic acid, 4-amino-3-[[4′-[(2,4-diaminophenyl)azo][1,1′-biphenyl]-4-yl]azo]-5-hydroxy-6-(phenylazo)-, disodium salt (Direct Black 38)
2150-54-1	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethyl[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[4,5-dihydroxy-, tetrasodium salt (Direct Blue 25)
2429-71-2	1-Naphthalenesulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[4-hydroxy-, disodium salt (Direct Blue 8)
2429-74-5	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-, tetrasodium salt (Direct Blue 15)
3701-40-4	2,7-Naphthalenedisulfonic acid, 4-hydroxy-3-[[4′-[(2-hydroxy-1-naphthalenyl)azo]-2,2′-dimethyl[1,1′-biphenyl]-4-yl]azo]-, disodium salt (Acid Red 99)
6358-57-2	2,7-Naphthalenedisulfonic acid, 3-[[2,2′-dimethyl-4′-[[4-[[(4-methylphenyl)sulfonyl]oxy]phenyl]azo][1,1′-biphenyl]-4-yl]azo]-4-hydroxy-, disodium salt (Acid Red 111)
6420-06-0	1-Naphthalenesulfonic acid, 4-hydroxy-3-[[4′-[(1-hydroxy-5-sulfo-2-naphthalenyl)azo]-

	3,3′-dimethyl[1,1′-biphenyl]-4-yl]azo]-, disodium salt (Direct Violet 28)
6420-22-0	2,7-Naphthalenedisulfonic acid, 5-amino-3-[[4′-[(6-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-3,3′-dimethyl[1,1′-biphenyl]-4-yl]azo]-4-hydroxy-, trisodium salt (Direct Blue 295)
6449-35-0	1-Naphthalenesulfonic acid, 3-[[4′-[(6-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-3,3′-dimethoxy[1,1′-biphenyl]-4-yl]azo]-4-hydroxy-, disodium salt (Direct Blue 151)
6459-94-5	1,3-Naphthalenedisulfonic acid, 8-[[3,3′-dimethyl-4′-[[4-[[(4-methylphenyl)sulfonyl]oxy]phenyl]azo][1,1′-biphenyl]-4-yl]azo]-7-hydroxy-, disodium salt (Acid Red 114)
6470-20-8	[1,1′-Biphenyl]-2,2′-disulfonic acid, 4-[(4,5-dihydro-3-methyl-5-oxo-1-phenyl-1H-pyrazol-4-yl)azo]-4′-[(2-hydroxy-1-naphthalenyl)azo]-, disodium salt (Acid Orange 56)
6548-29-4	2,7-Naphthalenedisulfonic acid, 4,4′-[(3,3′-dichloro[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[3-amino-, tetrasodium salt (Direct Red 46)
6548-30-7	1,3-Naphthalenedisulfonic acid, 8-[[3,3′-dimethoxy-4′-[[4-[[(4-methylphenyl)sulfonyl]oxy]phenyl]azo][1,1′-biphenyl]-4-yl]azo]-7-hydroxy-, disodium salt (Acid Red 128)
6655-95-4	Acetic acid, 2,2′-[[4,4′-bis[[1-hydroxy-6-[(4-methoxyphenyl)amino]-3-sulfo-2-naphthalenyl]azo][1,1′-biphenyl]-3,3′-diyl]bis(oxy)]bis-, tetrasodium salt (Direct Blue 158)
10169-02-5	[1,1’-Biphenyl]-2,2′-disulfonic acid, 4,4′-bis[(2-hydroxy-1-naphthalenyl)azo]-, disodium salt (Acid Red 97)
16071-86-6	Cuprate(2-), [5-[[4′-[[2,6-dihydroxy-3-[(2-hydroxy-5-sulfophenyl)azo]phenyl]azo][1,1′-biphenyl]-4-yl]azo]-2-hydroxybenzoato(4-)]-, disodium (Direct Brown 95)
67923-89-1	2,7-Naphthalenedisulfonic acid, 5-amino-4-hydroxy-3-[[4′-[(1-hydroxy-4-sulfo-2-naphthalenyl)azo]-3,3′-dimethoxy[1,1′-biphenyl]-4-yl]azo]-, trilithium salt
68318-35-4	2,7-Naphthalenedisulfonic acid, 4-amino-3-[[4′-[(2,4-dihydroxyphenyl)azo]-3,3′-dimethyl[1,1′-biphenyl]-4-yl]azo]-5-hydroxy-6-[(4-sulfophenyl)azo]-, trisodium salt
68400-36-2	2,7-Naphthalenedisulfonic acid, 4-amino-5-hydroxy-6-[[4′-[(4-hydroxyphenyl)azo]-3,3′-dimethyl[1,1′-biphenyl]-4-yl]azo]-3-[(4-nitrophenyl)azo]-, disodium salt
70210-28-5	Benzoic acid, 5-[[4′-[[6-amino-5-(1H-benzotriazol-5-ylazo)-1-hydroxy-3-sulfo-2-naphthalenyl]azo]-3,3′-dimethoxy[1,1′-biphenyl]-4-yl]azo]-2-hydroxy-4-methyl-, disodium salt
71215-83-3	Benzoic acid, 5-[[4′-[(2-amino-8-hydroxy-6-sulfo-1-naphthalenyl)azo]-2,2′-dichloro[1,1′-biphenyl]-4-yl]azo]-2-hydroxy-, disodium salt
71550-22-6	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-, tetralithium salt
72252-59-6	[1,1′-Biphenyl]-3,3′-dicarboxylic acid, 4-[[5-[[5-(aminosulfonyl)-2-hydroxyphenyl]azo]-1-hydroxy-6-(phenylamino)-3-sulfo-2-naphthalenyl]azo]-4′-[[1-[[(3-carboxy-4-hydroxyphenyl)amino]carbonyl]-2-oxopropyl]azo]-, tetrasodium salt
75659-72-2	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-, monolithium trisodium salt
75659-73-3	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-, dilithium disodium salt
75673-18-6	2,7-Naphthalenedisulfonic acid, 5-amino-4-hydroxy-3-[[4′-[(1-hydroxy-4-sulfo-2-naphthalenyl)azo]-3,3′-dimethoxy[1,1′-biphenyl]-4-yl]azo]-, monolithium disodium salt
75673-19-7	2,7-Naphthalenedisulfonic acid, 5-amino-4-hydroxy-3-[[4′-[(1-hydroxy-4-sulfo-2-naphthalenyl)azo]-3,3′-dimethoxy[1,1′-biphenyl]-4-yl]azo]-, dilithium monosodium salt
75673-34-6	1-Naphthalenesulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[4-hydroxy-, dilithium salt
75673-35-7	1-Naphthalenesulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-

	diyl)bis(azo)]bis[4-hydroxy-, monolithium monosodium salt
75752-17-9	2,7-Naphthalenedisulfonic acid, 3,3′-[(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis(azo)]bis[5-amino-4-hydroxy-, trilithium monosodium salt
83221-63-0	2,7-Naphthalenedisulfonic acid, 4-amino-3-[[4′-[(2,4-diaminophenyl)azo]-2,2′-disulfo[1,1′-biphenyl]-4-yl]azo]-5-hydroxy-6-(phenylazo)-, sodium salt
89923-60-4	Benzenesulfonic acid, 3,3′-[(2,2′-dimethyl[1,1′-biphenyl]-4,4′-diyl)bis[azo(4,5-dihydro-3-methyl-5-oxo-1H-pyrazole-4,1-diyl)]]bis[4-chloro-, disodium salt
93940-21-7	1-Triazene-1-carbonitrile, 3,3′-(3,3′-dimethoxy[1,1′-biphenyl]-4,4′-diyl)bis-

Table I-3: Benzidine Derivatives

CAS RN	DSL name (C.I. name)
91-97-4	1,1′-Biphenyl, 4,4′-diisocyanato-3,3′-dimethyl-
119-90-4	[1,1′-Biphenyl]-4,4′-diamine, 3,3′-dimethoxy- (Disperse Black 6)
119-93-7	[1,1′-Biphenyl]-4,4′-diamine, 3,3′-dimethyl- (Azoic Diazo Component 113)
366-29-0	[1,1′-Biphenyl]-4,4′-diamine, *N,N,N′,N′*-tetramethyl-
612-82-8	[1,1′-Biphenyl]-4,4′-diamine, 3,3′-dimethyl-, dihydrochloride

Table I-4: Monoazo Pigments

CAS RN	DSL name (C.I. name)
1103-38-4	1-Naphthalenesulfonic acid, 2-[(2-hydroxy-1-naphthalenyl)azo]-, barium salt (2:1) (Pigment Red 49:1)
2425-85-6	2-Naphthalenol, 1-[(4-methyl-2-nitrophenyl)azo]- (Pigment Red 3)
2512-29-0	Butanamide, 2-[(4-methyl-2-nitrophenyl)azo]-3-oxo-*N*-phenyl- (Pigment Yellow 1)
2786-76-7	2-Naphthalenecarboxamide, 4-[[4-(aminocarbonyl)phenyl]azo]-*N*-(2-ethoxyphenyl)-3-hydroxy- (Pigment Red 170)
2814-77-9	2-Naphthalenol, 1-[(2-chloro-4-nitrophenyl)azo]- (Pigment Red 4)
3468-63-1	2-Naphthalenol, 1-[(2,4-dinitrophenyl)azo]- (Pigment Orange 5)
5160-02-1	Benzenesulfonic acid, 5-chloro-2-[(2-hydroxy-1-naphthalenyl)azo]-4-methyl-, barium salt (2:1) (Pigment Red 53:1)
6372-81-2	Benzoic acid, 2-[(2-hydroxy-1-naphthalenyl)azo]-, barium salt (2:1) (Pigment Red 50:1)
6407-74-5	3H-Pyrazol-3-one, 4-[(2-chlorophenyl)azo]-2,4-dihydro-5-methyl-2-phenyl- (Pigment Yellow 60)
6410-09-9	2-Naphthalenol, 1-[(2-nitrophenyl)azo]- (Pigment Orange 2)
6410-13-5	2-Naphthalenol, 1-[(4-chloro-2-nitrophenyl)azo]- (Pigment Red 6)
6410-41-9	2-Naphthalenecarboxamide, *N*-(5-chloro-2,4-dimethoxyphenyl)-4-[[5-[(diethylamino)sulfonyl]-2-methoxyphenyl]azo]-3-hydroxy- (Pigment Red 5)
6417-83-0	2-Naphthalenecarboxylic acid, 3-hydroxy-4-[(1-sulfo-2-naphthalenyl)azo]-, calcium salt (1:1) (Pigment Red 63:1)
6486-23-3	Butanamide, 2-[(4-chloro-2-nitrophenyl)azo]-*N*-(2-chlorophenyl)-3-oxo- (Pigment Yellow 3)
6535-46-2	2-Naphthalenecarboxamide, 3-hydroxy-*N*-(2-methylphenyl)-4-[(2,4,5-trichlorophenyl)azo]- (Pigment Red 112)
7023-61-2	2-Naphthalenecarboxylic acid, 4-[(5-chloro-4-methyl-2-sulfophenyl)azo]-3-hydroxy-, calcium salt (1:1) (Pigment Red 48:2)
12236-62-3	Butanamide, 2-[(4-chloro-2-nitrophenyl)azo]-*N*-(2,3-dihydro-2-oxo-1H-benzimidazol-5-

	yl)-3-oxo- (Pigment Orange 36)
12236-64-5	2-Naphthalenecarboxamide, *N*-[4-(acetylamino)phenyl]-4-[[5-(aminocarbonyl)-2-chlorophenyl]azo]-3-hydroxy- (Pigment Orange 38)
12238-31-2	Pigment Red 52:2 (Pigment Red 52:2)
13515-40-7	Butanamide, 2-[(4-chloro-2-nitrophenyl)azo]-*N*-(2-methoxyphenyl)-3-oxo- (Pigment Yellow 73)
13824-00-5	2-Naphthalenecarboxamide, 3-hydroxy-*N*-(4-methoxyphenyl)-4-[(4-methylphenyl)azo]-
16403-84-2	2-Naphthalenecarboxamide, 4-[[5-(aminocarbonyl)-2-methylphenyl]azo]-3-hydroxy-*N*-phenyl- (Pigment Red 268)
17852-99-2	2-Naphthalenecarboxylic acid, 4-[(4-chloro-5-methyl-2-sulfophenyl)azo]-3-hydroxy-, calcium salt (1:1) (Pigment Red 52:1)
17947-32-9	2-Naphthalenecarboxamide, 3-hydroxy-*N*-(4-methoxyphenyl)-4-(phenylazo)-
36968-27-1	2-Naphthalenecarboxamide, 4-[[4-(aminocarbonyl)phenyl]azo]-3-hydroxy-*N*-(2-methoxyphenyl)- (Pigment Red 266)
43035-18-3	Benzenesulfonic acid, 4-[[3-[[2-hydroxy-3-[[(4-methoxyphenyl)amino]carbonyl]-1-naphthalenyl]azo]-4-methylbenzoyl]amino]-, calcium salt (2:1) (Pigment Red 247)
49744-28-7	2-Naphthalenol, 1-[(4-methoxy-2-nitrophenyl)azo]-
59487-23-9	2-Naphthalenecarboxamide, 4-[[5-[[[4-(aminocarbonyl)phenyl]amino]carbonyl]-2-methoxyphenyl]azo]-*N*-(5-chloro-2,4-dimethoxyphenyl)-3-hydroxy- (Pigment Red 187)
71832-83-2	2-Naphthalenecarboxylic acid, 4-[(5-chloro-4-methyl-2-sulfophenyl)azo]-3-hydroxy-, magnesium salt (1:1) (Pigment Red 48:5, Pigment Red 83:1)
74336-60-0	9,10-Anthracenedione, 1-[(5,7-dichloro-1,9-dihydro-2-methyl-9-oxopyrazolo[5,1-b]quinazolin-3-yl)azo]- (Pigment Red 251)
83249-60-9	1-Naphthalenesulfonic acid, 2-[(2-hydroxy-6-sulfo-1-naphthalenyl)azo]-, calcium salt (1:1)
85005-63-6	2-Naphthalenecarboxamide, 4-[(2,4-dinitrophenyl)azo]-3-hydroxy-*N*-phenyl-
94199-57-2	2-Naphthalenecarboxamide, *N*-(2-ethoxyphenyl)-3-hydroxy-4-[(2-nitrophenyl)azo]-

Table I-5: Azo Solvent Dyes

CAS RN	DSL name (C.I. name)
60-09-3	Benzenamine, 4-(phenylazo)- (Solvent Yellow 1)
60-11-7	Benzenamine, *N,N*-dimethyl-4-(phenylazo)- (Solvent Yellow 2)
85-83-6	2-Naphthalenol, 1-[[2-methyl-4-[(2-methylphenyl)azo]phenyl]azo]- (Solvent Red 24)
85-86-9	2-Naphthalenol, 1-[[4-(phenylazo)phenyl]azo]- (Solvent Red 23)
97-56-3	Benzenamine, 2-methyl-4-[(2-methylphenyl)azo]- (Solvent Yellow 3)
101-75-7	Benzenamine, *N*-phenyl-4-(phenylazo)-
103-33-3	Diazene, diphenyl-
495-54-5	1,3-Benzenediamine, 4-(phenylazo)- (Solvent Orange 3)
842-07-9	2-Naphthalenol, 1-(phenylazo)- (Disperse Yellow 97 , Solvent Yellow 14)
1229-55-6	2-Naphthalenol, 1-[(2-methoxyphenyl)azo]- (Solvent Red 1)
2646-17-5	2-Naphthalenol, 1-[(2-methylphenyl)azo]- (Solvent Orange 2)
2653-64-7	2-Naphthalenol, 1-(1-naphthalenylazo)- (Pigment Red 40, Solvent Red 4)
2832-40-8	Acetamide, *N*-[4-[(2-hydroxy-5-methylphenyl)azo]phenyl]- (Disperse Yellow 3, Solvent Yellow 77)
3118-97-6	2-Naphthalenol, 1-[(2,4-dimethylphenyl)azo]- (Solvent Orange 7)
5290-62-0	1-Naphthalenol, 4-[(4-nitrophenyl)azo]-
6368-72-5	2-Naphthalenamine, *N*-ethyl-1-[[4-(phenylazo)phenyl]azo]- (Solvent Red 19)

6407-78-9	3H-Pyrazol-3-one, 4-[(2,4-dimethylphenyl)azo]-2,4-dihydro-5-methyl-2-phenyl- (Solvent Yellow 18)
6535-42-8	1-Naphthalenol, 4-[(4-ethoxyphenyl)azo]- (Solvent Red 3)
21519-06-2	3H-Pyrazol-3-one, 2,4-dihydro-2-(3-hydroxyphenyl)-5-methyl-4-[[4-(phenylazo)phenyl]azo]-
73507-36-5	2-Naphthalenesulfonic acid, 7-(benzoylamino)-4-hydroxy-3-[[4-[(4-sulfophenyl)azo]phenyl]azo]-, compds. with *N,N′*-bis(mixed Ph and tolyl and xylyl)guanidine monohydrochloride (Solvent Red 33)
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-
85392-21-8	3-Pyridinecarbonitrile, 5-[[2-chloro-4-(phenylazo)phenyl]azo]-2,6-bis[(3-methoxypropyl)amino]-4-methyl-

Table I-6: Azo Disperse Dyes

CAS RN	DSL name (C.I. name)
2537-62-4	Acetamide, *N*-[2-[(2-bromo-6-cyano-4-nitrophenyl)azo]-5-(diethylamino)phenyl]-
3618-72-2	Acetamide, *N*-[5-[bis[2-(acetyloxy)ethyl]amino]-2-[(2-bromo-4,6-dinitrophenyl)azo]-4-methoxyphenyl]- (Disperse Blue 79:1)
5261-31-4	Propanenitrile, 3-[[2-(acetyloxy)ethyl][4-[(2,6-dichloro-4-nitrophenyl)azo]phenyl]amino]- (Disperse Orange 30)
6232-56-0	Ethanol, 2-[[4-[(2,6-dichloro-4-nitrophenyl)azo]phenyl]methylamino]- (Disperse Orange 5)
6250-23-3	Phenol, 4-[[4-(phenylazo)phenyl]azo]- (Disperse Yellow 23)
6300-37-4	Phenol, 2-methyl-4-[[4-(phenylazo)phenyl]azo]- (Disperse Yellow 7)
6657-00-7	Phenol, 4-[[2-methoxy-5-methyl-4-(phenylazo)phenyl]azo]-
12239-34-8	Acetamide, *N*-[5-[bis[2-(acetyloxy)ethyl]amino]-2-[(2-bromo-4,6-dinitrophenyl)azo]-4-ethoxyphenyl]- (Disperse Blue 79)
15958-27-7	Propanenitrile, 3-[[4-[(4-nitrophenyl)azo]phenyl][2-[[(phenylamino)carbonyl]oxy]ethyl]amino]-
16421-40-2	Acetamide, *N*-[5-[[2-(acetyloxy)ethyl](phenylmethyl)amino]-2-[(2-chloro-4,6-dinitrophenyl)azo]-4-methoxyphenyl]- (Disperse Blue 130)
16421-41-3	Acetamide, *N*-[5-[[2-(acetyloxy)ethyl](phenylmethyl)amino]-2-[(2,4-dinitrophenyl)azo]-4-methoxyphenyl]-
16586-42-8	Propanenitrile, 3-[ethyl[3-methyl-4-[(6-nitro-2-benzothiazolyl)azo]phenyl]amino]- (Disperse Violet 52, Disperse Red 179)
17464-91-4	Ethanol, 2,2′-[[4-[(2-bromo-6-chloro-4-nitrophenyl)azo]-3-chlorophenyl]imino]bis- (Disperse Brown 1:1)
19745-44-9	Propanenitrile, 3-[4-[(5-nitro-2-thiazolyl)azo](2-phenylethyl)amino]-
19800-42-1	Phenol, 4-[[2-methoxy-4-[(4-nitrophenyl)azo]phenyl]azo]- (Disperse Orange 29)
21811-64-3	Phenol, 4,4′-[1,4-phenylenebis(azo)]bis- (Disperse Yellow 68)
23355-64-8	Ethanol, 2,2′-[[3-chloro-4-[(2,6-dichloro-4-nitrophenyl)azo]phenyl]imino]bis- (Disperse Brown 1)
24610-00-2	Benzonitrile, 2-[[4-[(2-cyanoethyl)(2-phenylethyl)amino]phenyl]azo]-5-nitro-
25150-28-1	Propanenitrile, 3-[[4-[(6,7-dichloro-2-benzothiazolyl)azo]phenyl]ethylamino]-

25176-89-0	Propanenitrile, 3-[[4-[(5,6-dichloro-2-benzothiazolyl)azo]phenyl]ethylamino]-
26021-20-5	Acetamide, *N*-[2-[(2-bromo-4,6-dinitrophenyl)azo]-5-[(2-cyanoethyl)(2-hydroxyethyl)amino]-4-methoxyphenyl]- (Disperse Blue 94)
26850-12-4	Propanamide, *N*-[5-[bis[2-(acetyloxy)ethyl]amino]-2-[(2-chloro-4-nitrophenyl)azo]phenyl]- (Disperse Red 167)
27184-69-6	Phenol, 4,4'-[1,4-phenylenebis(azo)]bis[3-methyl-
28824-41-1	Propanenitrile, 3-[[4-[(4,6-dibromo-2-benzothiazolyl)azo]phenyl]ethylamino]-
29765-00-2	Benzamide, *N*-[5-[bis[2-(acetyloxy)ethyl]amino]-2-[(4-nitrophenyl)azo]phenyl]- (Disperse Red 135)
31030-27-0	Benzenamine, 4-[(2-chloro-4-nitrophenyl)azo]-*N*-ethyl-*N*-(2-phenoxyethyl)-
33979-43-0	Propanenitrile, 3-[[2-(acetyloxy)ethyl][4-[(5,6-dichloro-2-benzothiazolyl)azo]phenyl]amino]-
41362-82-7	Propanenitrile, 3-[[4-[(5,6-dichloro-2-benzothiazolyl)azo]phenyl]methylamino]-
42357-98-2	1H-Benz[de]isoquinoline-1,3(2H)-dione, 6-hydroxy-5-[(2-methoxy-4-nitrophenyl)azo]-2-methyl-
42358-36-1	1H-Benz[de]isoquinoline-1,3(2H)-dione, 2-ethyl-6-hydroxy-5-[(2-methoxy-4-nitrophenyl)azo]-
42852-92-6	Acetamide, *N*-[2-[(2-bromo-4,6-dinitrophenyl)azo]-4-methoxy-5-[(phenylmethyl)-2-propenylamino]phenyl]-
51249-07-1	3-Pyridinecarbonitrile, 1-(2-ethylhexyl)-1,2-dihydro-6-hydroxy-4-methyl-5-[(2-nitrophenyl)azo]-2-oxo-
52697-38-8	Acetamide, *N*-[2-[(2-bromo-4,6-dinitrophenyl)azo]-5-(diethylamino)phenyl]-
53950-33-7	Acetamide, *N*-[2-[(2-bromo-4,6-dinitrophenyl)azo]-5-[(2-cyanoethyl)amino]-4-methoxyphenyl]-
55252-53-4	Acetamide, *N*-[2-[(2-cyano-6-iodo-4-nitrophenyl)azo]-5-(diethylamino)phenyl]-
55281-26-0	Propanenitrile, 3-[[4-[(2,6-dibromo-4-nitrophenyl)azo]phenyl]ethylamino]- (Disperse Orange 61)
55290-62-5	Benzenesulfonamide, 4-[(1-butyl-5-cyano-1,6-dihydro-2-hydroxy-4-methyl-6-oxo-3-pyridinyl)azo]-*N*-(2-ethylhexyl)-
55619-18-6	Ethanol, 2,2′-[[4-[(2,6-dibromo-4-nitrophenyl)azo]phenyl]imino]bis-, diacetate (ester)
56532-53-7	Acetamide, *N*-[2-[(2,6-dicyano-4-nitrophenyl)azo]-5-(dipropylamino)phenyl]-
58104-55-5	2-Naphthalenesulfonamide, 6-hydroxy-*N*-(2-hydroxyethyl)-*N*-methyl-5-[[4-(phenylazo)phenyl]azo]-
59709-38-5	β-Alanine, *N*-[4-[(2-bromo-6-chloro-4-nitrophenyl)azo]phenyl]-*N*-(3-methoxy-3-oxopropyl)-, methyl ester
61799-13-1	3-Pyridinecarbonitrile, 5-[(2-cyano-4-nitrophenyl)azo]-2-[(2-hydroxyethyl)amino]-4-methyl-6-[[3-(2-phenoxyethoxy)propyl]amino]-
62531-00-7	Phenol, 4-[[4-(phenylazo)-1-naphthalenyl]azo]- (Disperse Orange 13)
63133-84-6	1(2H)-Quinolineethanol, 6-[(2-chloro-4,6-dinitrophenyl)azo]-3,4-dihydro-2,2,4,7-tetramethyl-
63134-15-6	Acetamide, *N*-[5-(dipropylamino)-2-[[5-(ethylthio)-1,3,4-thiadiazol-2-yl]azo]phenyl]- (Disperse Red 338)
63833-78-3	3-Pyridinecarbonitrile, 5-[(2-cyano-4-nitrophenyl)azo]-6-[(2-hydroxyethyl)amino]-4-methyl-2-[[3-(2-phenoxyethoxy)propyl]amino]-
64650-20-7	Carbamic acid, [4-[[4-[(4-hydroxyphenyl)azo]-2-methylphenyl]azo]phenyl]-, methyl ester
65122-05-6	Diazene, [(1,3-dihydro-1,1,3-trimethyl-2H-inden-2-ylidene)methyl](2-methoxyphenyl)-
66693-26-3	Propanamide, *N*-[5-[bis[2-(2-cyanoethoxy)ethyl]amino]-2-[(2-chloro-4,6-dinitrophenyl)azo]-4-methoxyphenyl]- (Disperse Blue 125)

67905-67-3	Propanenitrile, 3-[butyl[4-[(6-nitro-2-benzothiazolyl)azo]phenyl]amino]-
68214-63-1	3-Pyridinecarbonitrile, 5-[(3,4-dichlorophenyl)azo]-1,2-dihydro-6-hydroxy-4-methyl-2-oxo-1-(phenylamino)-
68214-66-4	Carbamic acid, [2-[(2-chloro-4-nitrophenyl)azo]-5-(diethylamino)phenyl]-, 2-ethoxyethyl ester
68516-64-3	Propanenitrile, 3-[[2-(acetyloxy)ethyl][4-[(2-chloro-4-nitrophenyl)azo]-3-methylphenyl]amino]-
68877-63-4	Acetamide, *N*-[2-[(2-bromo-4,6-dinitrophenyl)azo]-5-[(2-cyanoethyl)-2-propenylamino]-4-methoxyphenyl]-
68992-01-8	3-Pyridinecarbonitrile, 1-(2-ethylhexyl)-1,2-dihydro-6-hydroxy-5-[(4-methoxy-2-nitrophenyl)azo]-4-methyl-2-oxo-
69472-19-1	Propanenitrile, 3-[butyl[4-[(4-nitrophenyl)azo]phenyl]amino]-
70210-08-1	2-Naphthalenesulfonamide, *N*-[2-(acetyloxy)ethyl]-6-hydroxy-*N*-methyl-5-[[4-(phenylazo)phenyl]azo]- (Disperse Red 151)
70660-55-8	1-Naphthalenamine, 4-[(2-bromo-4,6-dinitrophenyl)azo]-*N*-(3-methoxypropyl)-
72828-63-8	Benzonitrile, 2-[[4-[[2-(acetyloxy)ethyl]butylamino]-2-methylphenyl]azo]-3-bromo-5-nitro-
72828-64-9	1,3-Benzenedicarbonitrile, 2-[[4-[[2-(acetyloxy)ethyl]butylamino]-2-methylphenyl]azo]-5-nitro- (Disperse Blue 287)
72927-94-7	Benzenamine, 4-[(2,6-dichloro-4-nitrophenyl)azo]-*N*-(4-nitrophenyl)-
72968-82-2	Methanesulfonamide, *N*-[2-[(2,6-dicyano-4-methylphenyl)azo]-5-(dipropylamino)phenyl]-
73003-64-2	2,4,10-Trioxa-7-azaundecan-11-oic acid, 7-[4-[(2,6-dichloro-4-nitrophenyl)azo]-3-methylphenyl]-3-oxo-, methyl ester
73398-96-6	3-Pyridinecarbonitrile, 5-[(9,10-dihydro-9,10-dioxo-1-anthracenyl)azo]-2,6-bis[(2-methoxyethyl)amino]-4-methyl- (Disperse Brown 21)
79542-46-4	Acetamide, *N*-[4-chloro-2-[2-(2-chloro-4-nitrophenyl)azo]-5-[(2-hydroxy-3-phenoxypropyl)amino]phenyl]- (Disperse Red 349)
83249-47-2	Acetamide, *N*-[2-[(2-bromo-6-cyano-4-nitrophenyl)azo]-5-(dipropylamino)phenyl]-
83249-49-4	Benzonitrile, 3-bromo-2-[[4-(diethylamino)-2-methylphenyl]azo]-5-methyl-
83249-53-0	Methanesulfonamide, *N*-[2-[(2-bromo-6-cyano-4-methylphenyl)azo]-5-(diethylamino)phenyl]-
83249-54-1	Methanesulfonamide, *N*-[2-[(2-bromo-6-cyano-4-methylphenyl)azo]-5-(dipropylamino)phenyl]-
90729-40-1	3-Pyridinecarbonitrile, 1-butyl-5-[[4-(4-chlorobenzoyl)-2-nitrophenyl]azo]-1,2-dihydro-6-hydroxy-4-methyl-2-oxo-
93805-00-6	Phenol, 4-[[2-methoxy-4-[(2-methoxyphenyl)azo]-5-methylphenyl]azo]-
106276-78-2	Benzoic acid, 2,3,4,5-tetrachloro-6-cyano-, methyl ester, reaction products with 4-[(4-aminophenyl)azo]-3-methylbenzenamine and sodium methoxide
127126-02-7	Propanenitrile, 3-[[2-(acetyloxy)ethyl][4-[(6,7-dichloro-2-benzothiazolyl)azo]phenyl]amino]-

Table I-7: Azo Acid Dyes

CAS RN	DSL name (C.I. name)
587-98-4	Benzenesulfonic acid, 3-[[4-(phenylamino)phenyl]azo]-, monosodium salt (Acid Yellow 36
633-96-5	Benzenesulfonic acid, 4-[(2-hydroxy-1-naphthalenyl)azo]-, monosodium salt (Acid

	Orange 7)
3071-73-6	1-Naphthalenesulfonic acid, 8-(phenylamino)-5-[[4-[(5-sulfo-1-naphthalenyl)azo]-1-naphthalenyl]azo]-, disodium salt (Acid Black 24)
6262-70-3	2-Naphthalenesulfonic acid, 6-hydroxy-5-[[4-[[4-(phenylamino)-3-sulfophenyl]azo]-1-naphthalenyl]azo]-, disodium salt (Acid Black 26)
6507-77-3	1,3-Naphthalenedisulfonic acid, 7-hydroxy-8-[[4-[1-[4-[(4-hydroxyphenyl)azo]phenyl]cyclohexyl]phenyl]azo]-, disodium salt (Acid Orange 33)
15792-43-5	2,7-Naphthalenedisulfonic acid, 5-(acetylamino)-3-[(4-dodecylphenyl)azo]-4-hydroxy-, disodium salt (Acid Red 138)
25317-22-0	1-Naphthalenesulfonic acid, 3-[[4-(benzoylethylamino)-2-methylphenyl]azo]-4-hydroxy-
29706-48-7	Benzenesulfonic acid, 3-[[[4-(2-benzothiazolylazo)-3-methylphenyl]ethylamino]methyl]-
33510-50-1	1-Naphthalenesulfonic acid, 8-(phenylamino)-5-[[4-[(3-sulfophenyl)azo]-1-naphthalenyl]azo]-, disodium salt (Acid Blue 113)
35342-16-6	7-Benzothiazolesulfonic acid, 2-[4-[(hexahydro-2,4,6-trioxo-5-pyrimidinyl)azo]phenyl]-6-methyl-, monolithium salt
51988-24-0	Benzenesulfonic acid, 3-[[4-[(4-hydroxy-3-methylphenyl)azo]-3-methoxyphenyl]azo]-, monolithium salt
52236-73-4	Benzenesulfonic acid, 4-[(5-amino-3-methyl-1-phenyl-1H-pyrazol-4-yl)azo]-2,5-dichloro-, monolithium salt
62133-79-3	2-Naphthalenesulfonic acid, 5-[[4-[ethyl[(3-sulfophenyl)methyl]amino]phenyl]azo]-8-(phenylazo)-, disodium salt
62133-80-6	2-Naphthalenesulfonic acid, 8-[[4-[ethyl[(3-sulfophenyl)methyl]amino]phenyl]azo]-5-(phenylazo)-, disodium salt
67892-55-1	1-Naphthalenesulfonic acid, 5-[[4-[(2-chlorophenyl)azo]-6(or 7)-sulfo-1-naphthalenyl]azo]-8-(phenylamino)-, disodium salt
68155-63-5	2,7-Naphthalenedisulfonic acid, 5-[[2,4-dihydroxy-5-[(4-nitrophenyl)azo]phenyl]azo]-4-hydroxy-3-[(2-hydroxy-3,5-dinitrophenyl)azo]-, disodium salt (Acid Brown 75)
68555-86-2	Benzenesulfonic acid, 4-[[5-methoxy-4-[(4-methoxyphenyl)azo]-2-methylphenyl]azo]-, sodium salt (Acid Orange 156)
70210-05-8	2,7-Naphthalenedisulfonic acid, 3-[[2,4-bis(2-methylphenoxy)phenyl]azo]-4-hydroxy-5-[[(4-methylphenyl)sulfonyl]amino]-, disodium salt (Acid Violet 54, Acid Violet 97)
70210-06-9	Benzenesulfonic acid, 3-[[ethyl[4-[[4-[(3-sulfophenyl)azo]-1-naphthalenyl]azo]phenyl]amino]methyl]-, disodium salt (Acid Red 119)
70210-25-2	2,7-Naphthalenedisulfonic acid, 5-[[2,4-dihydroxy-5-[(2-hydroxy-3,5-dinitrophenyl)azo]phenyl]azo]-4-hydroxy-3-[(4-nitrophenyl)azo]-, disodium salt
70210-34-3	2,7-Naphthalenedisulfonic acid, 5-[[2,4-dihydroxy-5-[[4-[(4-nitro-2-sulfophenyl)amino]phenyl]azo]phenyl]azo]-4-hydroxy-3-[[4-[(4-nitro-2-sulfophenyl)amino]phenyl]azo]-, tetrasodium salt (Acid Brown 58)
71720-89-3	2-Naphthalenesulfonic acid, 5-[[4-(4-cyclohexylphenoxy)-2-sulfophenyl]azo]-6-[(2,6-dimethylphenyl)amino]-4-hydroxy-, disodium salt
71873-51-3	Benzenesulfonic acid, 2,5-dichloro-4-[4-[[5-[[(dodecyloxy)carbonyl]amino]-2-sulfophenyl]azo]-4,5-dihydro-3-methyl-5-oxo-1H-pyrazol-1-yl]-, disodium salt (Acid Yellow 218)
72496-92-5	Naphthalenesulfonic acid, 5-[[2,4-dihydroxy-5-[[4-[(4-nitro-2-sulfophenyl)amino]phenyl]azo]phenyl]azo]-8-[[4-[(4-nitro-2-sulfophenyl)amino]phenyl]azo]-, trisodium salt (Acid Brown 194)
72828-67-2	1,3-Naphthalenedisulfonic acid, 7-hydroxy-8-[[4-[1-[4-[(4-hydroxyphenyl)azo]phenyl]cyclohexyl]phenyl]azo]-, potassium sodium salt

72828-83-2	2,7-Naphthalenedisulfonic acid, 5-(benzoylamino)-3-[[2-(2-cyclohexylphenoxy)phenyl]azo]-4-hydroxy-, disodium salt
72968-80-0	2-Naphthalenesulfonic acid, 5-[[4-[[(4-methylphenyl)sulfonyl]oxy]phenyl]azo]-8-[[4-[(4-nitro-2-sulfophenyl)amino]phenyl]azo]-, disodium salt
72968-81-1	2-Naphthalenesulfonic acid, 8-[[4-[[(4-methylphenyl)sulfonyl]oxy]phenyl]azo]-5-[[4-[(4-nitro-2-sulfophenyl)amino]phenyl]azo]-, disodium salt
72986-60-8	2-Naphthalenesulfonic acid, 5-[[4-[(4-nitro-2-sulfophenyl)amino]phenyl]azo]-8-[[4-[(phenylsulfonyl)oxy]phenyl]azo]-, disodium salt
72986-61-9	2-Naphthalenesulfonic acid, 8-[[4-[(4-nitro-2-sulfophenyl)amino]phenyl]azo]-5-[[4-[(phenylsulfonyl)oxy]phenyl]azo]-, disodium salt
75949-73-4	Benzenesulfonic acid, 4,4′-[methylenebis[4,1-phenyleneazo(4,5-dihydro-3-methyl-5-oxo-1*H*-pyrazole-4,1-diyl)]]bis[3-methyl-, disodium salt
79234-36-9	2,7-Naphthalenedisulfonic acid, 5-(benzoylamino)-3-[[2-(4-cyclohexylphenoxy)phenyl]azo]-4-hydroxy-, disodium salt
83006-48-8	Benzenesulfonic acid, 4-[4-[[3-[(ethylphenylamino)sulfonyl]-4-methylphenyl]azo]-4,5-dihydro-3-methyl-5-oxo-1H-pyrazol-1-yl]-
83006-74-0	1-Naphthalenesulfonic acid, 8-(phenylamino)-5-[[4-[(5-sulfo-1-naphthalenyl)azo]-1-naphthalenyl]azo]-, ammonium sodium salt
83006-77-3	1-Naphthalenesulfonic acid, 8-(phenylamino)-5-[[4-[(3-sulfophenyl)azo]-1-naphthalenyl]azo]-, ammonium sodium salt
83027-51-4	1,7-Naphthalenedisulfonic acid, 6-[[2-(4-cyclohexylphenoxy)phenyl]azo]-4-[[(2,4-dichlorophenoxy)acetyl]amino]-5-hydroxy-, disodium salt
83027-52-5	1,7-Naphthalenedisulfonic acid, 6-[[2-(2-cyclohexylphenoxy)phenyl]azo]-4-[[(2,4-dichlorophenoxy)acetyl]amino]-5-hydroxy-, disodium salt
83221-60-7	1,6-Naphthalenedisulfonic acid, 4-[[4-[[1-hydroxy-6-(phenylamino)-3-sulfo-2-naphthalenyl]azo]-1-naphthalenyl]azo]-, ammonium sodium salt
84559-92-2	2,7-Naphthalenedisulfonic acid, 3,3′-[azoxybis[(2-methoxy-4,1-phenylene)azo]]bis[4,5-dihydroxy-, tetralithium salt
84962-50-5	Benzenesulfonic acid, 2,5-dichloro-4-[[2-(dibutylamino)-4-methyl-6-[[2-(4-sulfophenyl)ethyl]amino]-5-pyrimidinyl]azo]-, sodium salt
85030-31-5	2,7-Naphthalenedisulfonic acid, 3-hydroxy-4-[[4-[[4-[(2-hydroxy-6-sulfo-1-naphthalenyl)azo]-2-methylphenyl]methyl]-3-methylphenyl]azo]-, sodium salt
85136-25-0	2,7-Naphthalenedisulfonic acid, 3,3′-[azoxybis[(2-methoxy-4,1-phenylene)azo]]bis[4,5-dihydroxy-, lithium sodium salt
85223-35-4 (102616-51-3*)	Benzoic acid, 3,3′-methylenebis[6-[[2,4-dihydroxy-5-[(4-sulfophenyl)azo]phenyl]azo]-, sodium salt
90218-20-5	Benzenesulfonic acid, 5-amino-2,4-dimethyl-, diazotized, coupled with diazotized 2,4-, 2,5-and 2,6-xylidine and 4-[(2,4-dihydroxyphenyl)azo]benzenesulfonic acid, sodium salts
90432-08-9	2,7-Naphthalenedisulfonic acid, 4-amino-5-hydroxy-, diazotized, coupled with diazotized 4-nitro-1,3-benzenediamine and resorcinol, potassium sodium salts
90459-02-2	2,7-Naphthalenedisulfonic acid, 5-amino-4-hydroxy-3-[[6-sulfo-4-[(4-sulfo-1-naphthalenyl)azo]-1-naphthalenyl]azo]-, diazotized, coupled with diazotized 4-nitrobenzenamine and resorcinol, potassium sodium salts
102616-51-3	Benzoic acid, 3,3′-methylenebis[6-[[2,4-dihydroxy-5-[(4-sulfonylphenyl)azo]phenyl]azo]-, sodium salt
114910-04-2	1-Naphthalenediazonium, 4-[[4-[(4-nitro-2-sulfophenyl)amino]phenyl]azo]-6-sulfo-, chloride, reaction products with formaldehyde and salicylic acid, ammonium sodium

	salts

* CAS RN 102616-51-3 has been removed from the CAS registry as it is the same as CAS RN 85223-35-4. Both CAS RNs belong to the same substance.

Table I-8: Azo Direct Dyes

CAS RN	DSL name (C.I. name)
915-67-3	2,7-Naphthalenedisulfonic acid, 3-hydroxy-4-[(4-sulfo-1-naphthalenyl)azo]-, trisodium salt (Food Red 9, Acid Red 27)
1325-37-7	C.I. Direct Yellow 11 (Direct Yellow 11)
1325-54-8	C.I. Direct Orange 39 (Direct Orange 39)
1934-21-0	1H-Pyrazole-3-carboxylic acid, 4,5-dihydro-5-oxo-1-(4-sulfophenyl)-4-[(4-sulfophenyl)azo]-, trisodium salt (Acid Yellow 23, Food Yellow 4)
2611-82-7	1,3-Naphthalenedisulfonic acid, 7-hydroxy-8-[(4-sulfo-1-naphthalenyl)azo]-, trisodium salt (Food Red 7, Acid Red 18)
2829-42-7	Benzoic acid, 3,3′-[carbonylbis(imino-4,1-phenyleneazo)]bis[6-hydroxy-, disodium salt (Direct Yellow 26)
2870-32-8	Benzenesulfonic acid, 2,2′-(1,2-ethenediyl)bis[5-[(4-ethoxyphenyl)azo]-, disodium salt (Direct Yellow 12)
3214-47-9	1,5-Naphthalenedisulfonic acid, 3,3′-[carbonylbis[imino(2-methyl-4,1-phenylene)azo]]bis-, tetrasodium salt (Direct Yellow 50)
3626-36-6	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-(phenylazo)-, disodium salt (Direct Orange 26)
3687-80-7	1-Naphthalenesulfonic acid, 4-[[1-hydroxy-6-[[[[5-hydroxy-6-[(2-methoxyphenyl)azo]-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-3-sulfo-2-naphthalenyl]azo]-, trisodium salt (Direct Red 26)
3761-53-3	2,7-Naphthalenedisulfonic acid, 4-[(2,4-dimethylphenyl)azo]-3-hydroxy-, disodium salt (Acid Red 26, Food Red 5)
4399-55-7	1,5-Naphthalenedisulfonic acid, 3-[[4-[[4-[(6-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-6-sulfo-1-naphthalenyl]azo]-1-naphthalenyl]azo]-, tetrasodium salt (Direct Blue 71)
5001-72-9	2-Naphthalenesulfonic acid, 7,7′-iminobis[4-hydroxy-3-(phenylazo)-, disodium salt (Direct Red 31)
5489-77-0	2-Naphthalenesulfonic acid, 3-[[4-[(2,4-dimethyl-6-sulfophenyl)azo]-2-methoxy-5-methylphenyl]azo]-4-hydroxy-7-(phenylamino)-, disodium salt (Direct Violet 51)
6406-87-7	2-Naphthalenesulfonic acid, 5-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-8-[[4-(phenylazo)-7-sulfo-1-naphthalenyl]azo]-, trisodium salt
6420-33-3	1,5-Naphthalenedisulfonic acid, 3,3′-[carbonylbis[imino(5-methoxy-2-methyl-4,1-phenylene)azo]]bis-, tetrasodium salt (Direct Yellow 34)
6420-41-3	2-Naphthalenesulfonic acid, 4-hydroxy-7-[[[[5-hydroxy-6-(phenylazo)-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-3-[(6-sulfo-2-naphthalenyl)azo]-, trisodium salt (Direct Red 4)
6420-43-5	2-Naphthalenesulfonic acid, 4-hydroxy-7-[[[[5-hydroxy-6-[(2-methylphenyl)azo]-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-3-[(2-methyl-4-sulfophenyl)azo]-, trisodium salt (Direct Red 62)
10114-47-3	7-Benzothiazolesulfonic acid, 2,2′-(azodi-4,1-phenylene)bis[6-methyl-, disodium salt
10134-33-5	2-Naphthalenesulfonic acid, 8-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-5-[[4-(phenylazo)-7-sulfo-1-naphthalenyl]azo]-, trisodium salt
10482-42-5	2-Naphthalenesulfonic acid, 5-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-8-[[4-

	(phenylazo)-6-sulfo-1-naphthalenyl]azo]-, trisodium salt
12217-64-0	1,3-Naphthalenedisulfonic acid, 7,7′-[carbonylbis[imino(5-methoxy-2-methyl-4,1-phenylene)azo]]bis-, tetrasodium salt (Direct Orange 72)
28706-21-0	1,3-Naphthalenedisulfonic acid, 7,7′-[iminobis[carbonyl(2-methyl-4,1-phenylene)azo]]bis-, tetrasodium salt
32829-81-5	Benzenesulfonic acid, 2,2′-(1,2-ethenediyl)bis[5-[[4-[(4-sulfophenyl)azo]phenyl]azo]-, tetrasodium salt
38801-08-0	Benzoic acid, 4,4′-[carbonylbis[imino(1-hydroxy-3-sulfo-6,2-naphthalenediyl)azo]]bis-, compd. with 2,2′,2′′-nitrilotris[ethanol] (1:4)
53523-90-3	Benzoic acid, 3,3′-[1,2-ethenediylbis[(3-sulfo-4,1-phenylene)azo]]bis[6-hydroxy-5-methyl-, tetralithium salt
64710-90-6	Benzoic acid, 5-[[4-[[4-[[4-[(4-amino-9,10-dihydro-9,10-dioxo-3-sulfo-1-anthracenyl)amino]-2-sulfophenyl]amino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]phenyl]azo]-2-hydroxy-, trisodium salt (Direct Green 28)
64761-00-4	2-Naphthalenesulfonic acid, 8-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-5-[[4-(phenylazo)-6-sulfo-1-naphthalenyl]azo]-, trisodium salt
65150-80-3	C.I. Direct Yellow 11, lithium salt (Direct Yellow 11, lithium salt)
71033-21-1	Benzothiazolesulfonic acid, 2,2′-(azodi-4,1-phenylene)bis[6-methyl-, disodium salt
71767-19-6	2-Naphthalenesulfonic acid, 5-[[6-amino-1-hydroxy-3-sulfo-5-[(3-sulfophenyl)azo]-2-naphthalenyl]azo]-6-methoxy-8-[[7-sulfo-4-[(3-sulfophenyl)azo]-1-naphthalenyl]azo]-, pentasodium salt
71873-49-9	Benzoic acid, 4,4′-[1,2-ethenediylbis[(3-sulfo-4,1-phenylene)-*ONN*-azoxy-4,1-phenyleneazo]]bis-, tetrasodium salt
72139-21-0	Benzoic acid, 3,3′-[(1,4-dioxo-2-butene-1,4-diyl)bis(imino-4,1-phenyleneazo)]bis[6-hydroxy-, disodium salt
72152-50-2	Benzoic acid, 2-[[6-[[4-[[6-(benzoylamino)-1-hydroxy-3-sulfo-2-naphthalenyl]azo]-3-methylbenzoyl]amino]-1-hydroxy-3-sulfo-2-naphthalenyl]azo]-, trisodium salt
72245-49-9	Benzoic acid, 4-[[1-hydroxy-6-[[[[5-hydroxy-6-[(2-methyl-4-sulfophenyl)azo]-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-3-sulfo-2-naphthalenyl]azo]-, sodium salt
72245-56-8	2,7-Naphthalenedisulfonic acid, 4-amino-3-[[4-[[[4-[(2,4-diaminophenyl)azo]phenyl]amino]carbonyl]phenyl]azo]-5-hydroxy-6-(phenylazo)-, sodium salt
72749-87-2	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-[(2-methylphenyl)azo]-, disodium salt
72749-88-3	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-[(2-methoxyphenyl)azo]-, disodium salt
72869-93-3	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-[(6-sulfo-2-naphthalenyl)azo]-, compd. with 2,2′-(methylimino)bis[ethanol] (1:4)
75150-14-0	1,4-Benzenedisulfonic acid, 2-[[4-[[4-[[1-hydroxy-6-(phenylamino)-3-sulfo-2-naphthalenyl]azo]-1-naphthalenyl]azo]-6-sulfo-1-naphthalenyl]azo]-, ammonium sodium salt
75768-93-3	2-Naphthalenesulfonic acid, 7-(benzoylamino)-4-hydroxy-3-[[4-[(4-sulfophenyl)azo]phenyl]azo]-, compd. with 2,2′,2′′-nitrilotris[ethanol] (1:2) (Direct Red 81, triethanolamine salt)
83221-53-8	Benzoic acid, 5-[[4-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-1-naphthalenyl]azo]-2-hydroxy-, sodium salt
83221-54-9	Benzoic acid, 3-[[4-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-1-

	naphthalenyl]azo]-2-hydroxy-, sodium salt
83221-56-1	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-(phenylazo)-, sodium salt
83221-68-5	2-Naphthalenesulfonic acid, 6-[(2,4-diaminophenyl)azo]-3-[[4-[[4-[[7-[(2,4-diaminophenyl)azo]-1-hydroxy-3-sulfo-2-naphthalenyl]azo]phenyl]amino]-3-sulfophenyl]azo]-4-hydroxy-, trilithium salt
83221-69-6	2-Naphthalenesulfonic acid, 6-[(2,4-diaminophenyl)azo]-3-[[4-[[4-[[7-[(2,4-diaminophenyl)azo]-1-hydroxy-3-sulfo-2-naphthalenyl]azo]phenyl]amino]-3-sulfophenyl]azo]-4-hydroxy-, lithium sodium salt
83221-72-1	2,7-Naphthalenedisulfonic acid, 4-amino-3,6-bis[[4-[(2,4-diaminophenyl)azo]phenyl]azo]-5-hydroxy-, lithium sodium salt
83221-73-2	Benzoic acid, 4,4′-[carbonylbis[imino(1-hydroxy-3-sulfo-6,2-naphthalenediyl)azo]]bis-, sodium salt
83221-74-3	Benzoic acid, 4-[[1-hydroxy-6-[[[[5-hydroxy-6-(phenylazo)-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-3-sulfo-2-naphthalenyl]azo]-, sodium salt
83232-28-4	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[3-[[4-(acetylamino)phenyl]azo]-4-hydroxy-, sodium salt
83232-29-5	2-Naphthalenesulfonic acid, 3-[[4-(acetylamino)phenyl]azo]-4-hydroxy-7-[[[[5-hydroxy-6-(phenylazo)-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-, sodium salt
83232-30-8	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-[(2-methylphenyl)azo]-, sodium salt
83232-31-9	2-Naphthalenesulfonic acid, 7,7′-(carbonyldiimino)bis[4-hydroxy-3-[(2-methyl-4-sulfophenyl)azo]-, sodium salt
83232-32-0	2-Naphthalenesulfonic acid, 4-hydroxy-7-[[[[5-hydroxy-6-[(2-methylphenyl)azo]-7-sulfo-2-naphthalenyl]amino]carbonyl]amino]-3-[(2-methyl-4-sulfophenyl)azo]-, sodium salt
83783-94-2	2,7-Naphthalenedisulfonic acid, 3,3′-[1,2-ethenediylbis[(3-sulfo-4,1-phenylene)azo]]bis[5-amino-4-hydroxy-, lithium sodium salt, compd. with 2,2′-(methylimino)bis[ethanol]
83783-95-3	2-Naphthalenesulfonic acid, 3,3′-[1,2-ethenediylbis[(3-sulfo-4,1-phenylene)azo]]bis[6-amino-4-hydroxy-, lithium sodium salt, compd. with 2,2′-(methylimino)bis[ethanol]
83783-96-4	2,7-Naphthalenedisulfonic acid, 5-amino-3-[[4-[2-[4-[(7-amino-1-hydroxy-3-sulfo-2-naphthalenyl)azo]-2-sulfophenyl]ethenyl]-3-sulfophenyl]azo]-4-hydroxy-, lithium sodium salt, compd. with 2,2′-(methylimino)bis[ethanol]
83783-99-7	Benzoic acid, 3,3′-[1,2-ethenediylbis[(3-sulfo-4,1-phenylene)azo]]bis[6-hydroxy-5-methyl-, lithium sodium salt, compd. with 2,2′-(methylimino)bis[ethanol]
84878-16-0	2,7-Naphthalenedisulfonic acid, 4-amino-6-[[4-[[4-[(2,4-dihydroxyphenyl)azo]phenyl]thio]phenyl]azo]-5-hydroxy-3-[(4-nitrophenyl)azo]-, sodium salt
84878-17-1	2,7-Naphthalenedisulfonic acid, 4-amino-6-[[4-[[[4-[(2,4-dihydroxyphenyl)azo]phenyl]amino]sulfonyl]phenyl]azo]-5-hydroxy-3-[(4-nitrophenyl)azo]-, potassium salt
85169-18-2	Glycine, N-[4-[[2-[4-[[1-amino-8-hydroxy-7-(phenylazo)-3,6-disulfo-2-naphthalenyl]azo]phenyl]-1H-benzimidazol-5-yl]azo]-3-hydroxyphenyl]-, compd. with 2,2’-iminobis[ethanol] (1:3)
85269-31-4	Benzoic acid, 3,3′-[1,2-ethenediylbis[(3-sulfo-4,1-phenylene)azo]]bis[6-hydroxy-5-methyl-, potassium salt, compd. with 2,2′,2′ ′-nitrilotris[ethanol]
93803-37-3	2,7-Naphthalenedisulfonic acid, 4-amino-5-hydroxy-3-[[4-[5-[(4-hydroxyphenyl)azo]-1H-benzimidazol-2-yl]phenyl]azo]-6-(phenylazo)-, disodium salt

102082-94-0	2,7-Naphthalenedisulfonic acid, 4-amino-6-[[4-[[[4-[(2,4-diaminophenyl)azo]phenyl]amino]sulfonyl]phenyl]azo]-5-hydroxy-3-[(4-nitrophenyl)azo]-, lithium salt
110152-63-1	Benzenesulfonic acid, 2,2′-(1,2-ethenediyl)bis[5-[(4-hydroxyphenyl)azo]-, lithium sodium salt

Table I-9: Azo Reactive Dyes

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

Table I-10: Azo Basic Dyes

CAS RN	DSL name (C.I. name)
136-40-3	2,6-Pyridinediamine, 3-(phenylazo)-, monohydrochloride
532-82-1	1,3-Benzenediamine, 4-(phenylazo)-, monohydrochloride (Basic Orange 2)
2869-83-2	Phenazinium, 3-(diethylamino)-7-[[4-(dimethylamino)phenyl]azo]-5-phenyl-, chloride
4608-12-2	Phenazinium, 3-(dimethylamino)-7-[[4-(dimethylamino)phenyl]azo]-5-phenyl-, chloride
4618-88-6	Phenazinium, 3-amino-7-[[4-(dimethylamino)phenyl]azo]-5-phenyl-, chloride
10189-42-1	Pyridinium, 1-[2-[[4-[[2,6-dichloro-4-[(dimethylamino)sulfonyl]phenyl]azo]phenyl]ethylamino]ethyl]-, chloride
14408-20-9	Pyridinium, 1-[2-[[4-[(2,6-dichloro-4-nitrophenyl)azo]phenyl]ethylamino]ethyl]-, chloride
14970-39-9	1H-1,2,4-Triazolium, 5-[[4-(diethylamino)phenyl]azo]-1,4-dimethyl-,trichlorozincate(1-)
23408-72-2	Benzothiazolium, 2-[[4-(dimethylamino)phenyl]azo]-3-ethyl-6-methoxy-, trichlorozincate(1-)
29508-48-3	1H-Pyrazolium, 1,5-dimethyl-3-[(2-methyl-1H-indol-3-yl)azo]-2-phenyl-, methyl sulfate
36986-04-6	Pyridinium, 1-[2-[[4-[(2-chloro-4-nitrophenyl)azo]phenyl]ethylamino]ethyl]-, chloride
52769-39-8	1H-1,2,4-Triazolium, dimethyl-3-[[4-[methyl(phenylmethyl)amino]phenyl]azo]-, trichlorozincate(1-)
59709-10-3	Pyridinium, 1-[2-[[4-[(2-chloro-4-nitrophenyl)azo]phenyl]ethylamino]ethyl]-, acetate

63589-49-1	1H-Pyrazolium, 2-cyclohexyl-3-[[4-(diethylamino)phenyl]azo]-1-methyl-, (T-4)-tetrachlorozincate(2-) (2:1)
63681-54-9	Benzenesulfonic acid, dodecyl-, compd. with 4-(phenylazo)-1,3-benzenediamine (1:1)
65150-98-3	Thiazolium, 2-[[4-(diethylamino)phenyl]azo]-3-methyl-, (T-4)-tetrachlorozincate(2-) (2:1)
68929-07-7	Benzothiazolium, 2-[[4-[ethyl(2-hydroxyethyl)amino]phenyl]azo]-5-methoxy-3-methyl-, methyl sulfate (salt)
68936-17-4	1H-Imidazolium, 2-[[4-(dimethylamino)phenyl]azo]-1,3-dimethyl-, (T-4)-tetrachlorozincate(2-) (2:1)
69852-41-1	Benzothiazolium, 2-[[4-[ethyl(2-hydroxyethyl)amino]phenyl]azo]-6-methoxy-3-methyl-, (T-4)-tetrachlorozincate(2-) (2:1)
71032-95-6	2-Naphthalenesulfonic acid, 7-[[4,6-bis[[3-(diethylamino)propyl]amino]-1,3,5-triazin-2-yl]amino]-4-hydroxy-3-[[4-(phenylazo)phenyl]azo]-, monoacetate (salt)
72361-40-1	Pyridinium, 1-[2-[[4-[(2-bromo-4,6-dinitrophenyl)azo]-3-methylphenyl]ethylamino]ethyl]-, chloride
72379-36-3	1H-1,2,4-Triazolium, 5-[[4-[ethyl(phenylmethyl)amino]phenyl]azo]-1,4-dimethyl-, (T-4)-tetrachlorozincate(2-) (2:1)
72379-37-4	1H-1,2,4-Triazolium, 3-[[4-[ethyl(phenylmethyl)amino]phenyl]azo]-1,2-dimethyl-, (T-4)-tetrachlorozincate(2-) (2:1)
74744-63-1	1H-1,2,4-Triazolium, 3,3′ (or 5,5′)-[1,2-ethanediylbis[(ethylimino)-4,1-phenyleneazo]]bis[1,4-dimethyl-, (T-4)-tetrachlorozincate(2-) (1:1)
75199-20-1	1,3′-Bipyridinium, 1′,2′-dihydro-6′-hydroxy-3,4′-dimethyl-2′-oxo-5′-[[4-(phenylazo)phenyl]azo]-, chloride
75660-25-2	1,3-Benzenediamine, 4-(phenylazo)-, monoacetate
79234-33-6	1,3-Benzenediamine, 4-(phenylazo)-, acetate
83969-13-5	1,3,4-Thiadiazolium, 5-[bis(1-methylethyl)amino]-2-[[4-(dimethylamino)phenyl]azo]-3-methyl-, sulfate (2:1)
85114-37-0	1H-1,2,4-Triazolium, 1,4-dimethyl-3(or 5)-[[4-[methyl(phenylmethyl)amino]phenyl]azo]-, (T-4)-tetrachlorozincate(2-) (2:1)
85480-88-2	Benzothiazolium, 3-(3-amino-3-oxopropyl)-2-[(1-ethyl-2-phenyl-1H-indol-3-yl)azo]-, (T-4)-tetrachlorozincate(2-) (2:1)
93783-70-1	1,3,4-Thiadiazolium, 5-[bis(1-methylethyl)amino]-2-[[4-(dimethylamino)phenyl]azo]-3-methyl-, trichlorozincate(1-)
10114-58-6	1,3-Benzenediamine, 4,4′-[1,3-phenylenebis(azo)]bis-, dihydrochloride (Basic Brown 1, dihydrochloride)
125329-01-3	Propanoic acid, 2-hydroxy-, compd. with 7-[[4,6-bis[[3-(diethylamino)propyl]amino]-1,3,5-triazin-2-yl]amino]-4-hydroxy-3-[[4-(phenylazo)phenyl]azo]-2-naphthalenesulfonic acid (1:1)

Table I-11: Azo Mordant Dyes

CAS RN	DSL name (C.I. name)
85029-57-8	Amines, C10–14-branched and linear alkyl, bis[2,4-dihydro-4-[(2-hydroxy-4-nitrophenyl)azo]-5-methyl-2-phenyl-3H-pyrazol-3-onato(2-)]chromate(1-)
94276-35-4	Xanthylium, 9-[2-(ethoxycarbonyl)phenyl]-3,6-bis(ethylamino)-2,7-dimethyl-, hydroxy[2-hydroxy-5-nitro-3-[[2-oxo-1-[(phenylamino)carbonyl]propyl]azo]benzenesulfonato(3-)]chromate(1-)

Table I-12: Azo Food Dyes

CAS RN	DSL name (C.I. name)
106028-58-4	2,7-Naphthalenedisulfonic acid, 6-amino-4-hydroxy-3-[[7-sulfo-4-[(4-sulfophenyl)azo]-1-naphtalenyl]azo]-, tetralithium salt (Food Black 2, lithium salt)

Table I-13: Unknowns

CAS RN	DSL name (C.I. name)
6708-61-8	1-Triazene, 1-(4-nitro-1-naphthalenyl)-3-[4-(phenylazo)phenyl]-
63224-47-5	Benzenediazonium, 4-[(2,6-dichloro-4-nitrophenyl)azo]-2,5-dimethoxy-, (T-4)-tetrachlorozincate(2-) (2:1)
63281-10-7	3-Pyridinecarbonitrile, 5-[[2-chloro-4-(methylsulfonyl)phenyl]azo]-4-methyl-2,6-bis[[3-(2-phenoxyethoxy)propyl]amino]-
72391-06-1	Spiro[isobenzofuran-1(3H),9′ (8′aH)-xanthylium], 3′,6′-bis(diethylamino)-3-oxo-, chloride, compd. with [4-[(4,5-dihydro-3-methyl-5-oxo-1-phenyl-1H-pyrazol-4-yl)azo]-3-hydroxy-1-naphthalenesulfonato(3-)]chromium (1:1)
83221-38-9	Benzenesulfonamide, 4-[[4-[[4-(2-hydroxybutoxy)-3-methylphenyl]azo]phenyl]amino]-3-nitro-*N*-(phenylsulfonyl)-, monolithium salt

Table I-14: Aromatic Amines

CAS RN	DSL name (C.I. name)
88-53-9	Benzenesulfonic acid, 2-amino-5-chloro-4-methyl-
90-04-0	Benzenamine, 2-methoxy-
91-59-8	2-Naphthalenamine
95-51-2	Benzenamine, 2-chloro-
95-53-4	Benzenamine, 2-methyl-
95-76-1	Benzenamine, 3,4-dichloro-
95-80-7	1,3-Benzenediamine, 4-methyl- (Oxidation Base 35)
100-01-6	Benzenamine, 4-nitro- (Azoic Diazo Component 37, Developer 17)
101-14-4	Benzenamine, 4,4′-methylenebis[2-chloro-
101-77-9	Benzenamine, 4,4′-methylenebis-
106-47-8	Benzenamine, 4-chloro-
106-49-0	Benzenamine, 4-methyl- (Azoic Coupling Component 107)
108-45-2	1,3-Benzenediamine (Developer 11)
123-30-8	Phenol, 4-amino- (Oxidation Base 6)
156-43-4	Benzenamine, 4-ethoxy-
540-23-8	Benzenamine, 4-methyl-, hydrochloride
541-69-5	1,3-Benzenediamine, dihydrochloride
615-05-4	1,3-Benzenediamine, 4-methoxy- (Oxidation Base 12)