3

Toxicology of textile dyes

P. GREGORY, Avecia, UK

3.1 Introduction

This chapter addresses the toxicology of textile dyes. Section 3.2 takes a brief look at the historical aspects, particularly around the mid-twentieth century when a link between dyes (and their intermediates) and bladder cancer in textile workers became apparent.

In Section 3.3, the acute (short-term) toxicological effects of textile dyes are discussed. The short-term problems are skin irritation and skin sensitisation, caused primarily by reactive dyes for cotton and viscose, and disperse dyes for polyester, polyamide and acetate rayon.

The main part of the chapter, Section 3.4, is concerned with the chronic (long-term) effects of textile dyes. Carcinogenicity (cancer-causing) is the main chronic effect and this is covered in detail. The known data is reviewed and structure–carcinogenicity relationships for textile dyes, particularly the most important class, azo dyes, are discussed. Other dye classes, such as anthraquinone dyes and cationic (basic) dyes, as well as the building blocks of dyes, the chemical intermediates, are also covered. The mode of action of carcinogenic dyes and their metabolites are elucidated and ways to avoid and eliminate carcinogenicity in textile dyes are presented. The section ends with a look at metal complex dyes and the toxicological implications of metals.

Section 3.5 considers future trends for textile dyes in relation to toxicology. This includes the design of safer dyes by utilising the extensive and ever-increasing knowledge of the relationships between the structure of dyes and toxicity, cleaner dyes, and by having more consideration regarding the formation of toxic products during the degradation of waste dyes in effluent treatment plants. The role of natural dyes is also discussed.

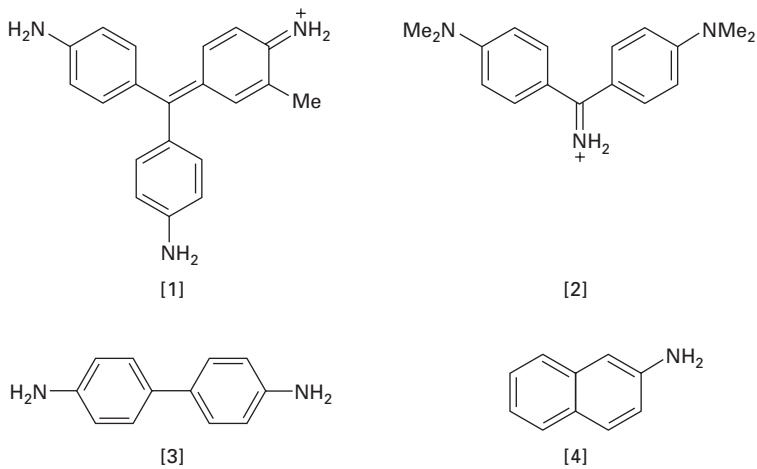
Finally, Section 3.6 concludes the chapter by presenting sources of further information and advice regarding the toxicology of textile dyes.

3.2 Historical aspects

Colorants have been used by mankind for many thousands of years. The earliest known use of a colorant was by Neanderthal man about 180 000 years ago. They used red ochre (essentially iron oxide), an inorganic pigment obtained from riverbeds, to daub the bodies of the dead before burial. The first known use of an organic colorant was much later, *c*. 4000 years ago, when the blue dye indigo was found in the wrappings of mummies in Egyptian tombs (Gordon, 1983). It is highly unlikely that either Neanderthal man or the ancient Egyptians considered the toxicological aspects of the colorants they used.

Until the late nineteenth century, all the colorants were obtained from nature. The main sources of natural dyes were plants, but insects and molluscs were also used. Vast amounts of raw materials were required to produce a tiny amount of impure dye and the process was land and labour intensive (Gordon, 1983). This meant that the workers involved in obtaining natural dyes were generally only exposed to dilute amounts of the dye. Furthermore, they never had to handle any chemical intermediates to synthesise the dyes, unlike their modern counterparts.

It was not until after Perkin's historic discovery of the first synthetic dye, mauveine, in 1856, that dyes (and later pigments) were manufactured on a large scale. The workers involved in the manufacture of dyes became exposed not only to the dyes themselves but also to the chemical intermediates used in their manufacture. Many years later, it became apparent that workers involved in the manufacture of certain dyes, such as fuchsine (see Fig. 3.1, C.I. Basic Violet 14 [1]) and auramine (C.I. Basic Yellow 2 [2]), and particularly



3.1 Structures of fuchsine (C.I. Basic Violet 14 [1]), auramine (C.I. Basic Yellow 2 [2]), benzidine [3] and 2-naphthylamine [4].

dyes based on benzidine [3] and 2-naphthylamine [4], developed a high incidence of bladder cancer (Hunger, 2003). It was established later that both benzidine and 2-naphthylamine are indeed human bladder carcinogens. Once this information was known, all responsible dye manufacturers took action to cease production of these proven human carcinogens and any dyes using them. It is to its eternal credit that the colorant manufacturing industry of Western Europe began to investigate the toxicological and ecotoxicological properties of dyes (and pigments) long before chemical and environmental regulations existed. Thus, in 1974, the member companies of ETAD (Ecological and Toxicological Association of Dyes and Organic Pigment Manufacturers) voluntarily developed Safety Data Sheets with appropriate information on the hazardous potential of colorants. Nowadays, the concept of Safety Data Sheets has spread worldwide (Hunger, 2003).

The world production of colorants is *c*. 1 million tonnes per year, of which *c*. 50% are textile dyes (Nousiainen, 1997). Textile dyes are therefore very important. They are also ubiquitous, being encountered in almost every aspect of our daily lives. For example, we are constantly in direct contact with textile dyes because of the clothes we wear, and in indirect contact with them because of furnishings, such as bedding, carpets, curtains, lounge suites and car seats. Therefore, it is imperative that textile dyes are non-toxic and safe. To ensure this is the case, very strict test protocols exist which every textile dye must pass before it is allowed on to the marketplace. Currently, the three main regulatory bodies worldwide are the European Inventory of Existing Commercial Substances (EINECS), the Toxic Substances Control Act (TSCA) in the USA, and the Ministry of Technology and Industry (MITI) in Japan (Hunger, 1991).

For registration of a textile dye in the European Union, a registration package is required which includes:

1. Identity of the substance
2. Information on the substance
3. Physico-chemical properties of the substance
4. Toxicological studies
5. Eco-toxicological studies.

It is the toxicological aspects of textile dyes that are discussed in this chapter. These may be divided into acute, or short-term effects and chronic, or long-term effects.

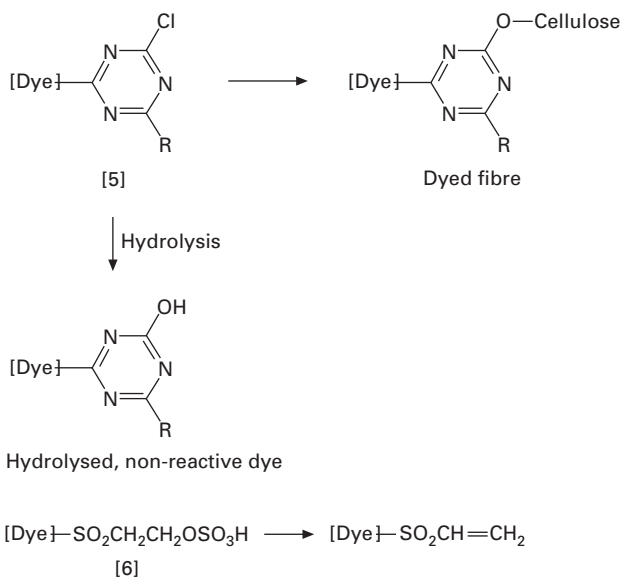
3.3 Acute toxicity of textile dyes

Acute toxicity involves oral ingestion and inhalation, skin and eye irritation, and skin sensitisation. The main problems of acute toxicity with textile dyes are skin irritation and skin sensitisation, caused mainly by reactive dyes for

cotton and viscose, and disperse dyes for polyester, polyamide and acetate rayon. A comprehensive review of acute toxicity data, including skin and eye irritation of numerous commercial dyes, obtained from Safety Data Sheets, revealed that the potential for these acute toxic effects was very low (Anliker, 1979). However, dermatologists have reported skin reactions thought to be caused by reactive dyes and disperse dyes (Hatch, 1984, 1986, 1998, 1999; Pratt, 2000; Tronnier, 2002).

Reactive dyes for cotton are water-soluble dyes, which contain a group capable of forming a covalent bond with the hydroxyl groups in the cellulose polymer during the dyeing process. The two main reactive groups, as shown in Fig. 3.2, are the monochlorotriazinyl (MCT) group [5] and the *beta*-sulphatoethylsulphone [masked vinyl sulphone (VS)] group [6], either alone or in combination (Gordon, 1983). Once the reactive dye has been used to colour the cellulosic fabric, no reactive dye should remain. The reactive dye is bound to the fibre with a covalent ether bond and any reactive dye that did not become attached to the fibre will have been hydrolysed in the dyeing process and removed in the dyebath effluent. Therefore, fabrics dyed with reactive dyes should pose no problems for the end-user of the product, the general public.

Reactive dyes can, however, cause problems in plant workers who manufacture the dyes and textile workers who handle the dyes in the dyeing process. There is evidence that some reactive dyes cause contact dermatitis, allergic conjunctivitis, rhinitis, occupational asthma or other allergic reactions



3.2 The fate of reactive dyes in the dyeing process.

in such workers. The problem is caused by the ability of reactive dyes to combine with human serum albumin (HSA) to give a dye-HSA conjugate, which acts as an antigen. The antigen produces specific immunoglobulin E (IgE) and, through the release of chemicals such as histamine, causes allergic reactions (Hunger, 2003; Luczynska, 1986). A study done in 1985 of 414 workers, such as dye-house operators, dye-store workers, mixers, weighers and laboratory staff, who were exposed to reactive dye powders, found that 21 of them were identified as having allergic reactions, including occupational asthma, due to one or more reactive dyes (Hunger, 2003; Platzek, 1997).

A list of reactive dyes that have caused respiratory or skin sensitisation in workers on occupational exposure has been compiled by ETAD (Table 3.1) (Hunger, 2003; Motschi, 2000). In order to minimise the risk from reactive dyes, exposure to dye dust should be avoided. This may be achieved by using liquid dyes, low dusting formulations and by using the appropriate personal protective equipment. As mentioned earlier, after dyeing and fixation,

Table 3.1 Reactive dyes classified as respiratory/skin sensitisers

C.I.* name	C.I. no.	CAS† no.
Reactive Yellow 25		[72139-14-1](3Na)
Reactive Yellow 39	18971	[70247-70-0](2Na)
Reactive Yellow 175		[111850-27-2](2Na)
Reactive Orange 4	18260	[70616-90-9](3Na)
Reactive Orange 12	13248	[70161-14-7](3Na)
		[93658-87-8](xNa)
Reactive Orange 14		[12225-86-4](acid)
Reactive Orange 16		[20262-58-2](2Na)
		[106027-83-2](2Li)
Reactive Orange 64		[83763-57-9](xNa)
Reactive Orange 67		[83763-54-6](xNa)
Reactive Orange 86		[57359-00-9](3Na)
Reactive Orange 91		[63817-39-0](3Na)
Reactive Red 29		[94006-25-4](5Na)
		[70865-39-3](4Na)
Reactive Red 65		[70210-40-1](2Na)
Reactive Red 66	17555	[70210-39-8[(2Na)
Reactive Red 123		[85391-83-9](xNa)
		[68959-17-1](2Na)
Reactive Red 219		[149057-72-7](4Na)
Reactive Red 225		[83399-95-5](xNa)
Reactive Violet 33		[69121-25-1](3Na)
Reactive Blue 114		[72139-17-4](2Na)
Reactive Blue 204		[85153-92-0](6Na)
Reactive Black 5	20505	[17095-24-8](4Na)

*Colour Index, a comprehensive listing of the tradenames, properties and structures, if known, of all commercial dyes and pigments.
†Chemical Abstract Services.

reactive dyes have completely different toxicological properties because the reactive group is no longer present and the high water-fastness of the dyed fabric ensures that no dye is exposed to the skin of the wearer. Consequently, no cases of allergic reactions have been reported by consumers wearing textiles dyed with reactive dyes (Hunger, 2003).

Certain disperse dyes have been implicated in causing allergic reactions, particularly when they are used for skin-tight, close-fitting clothes made from synthetic fibres. The sweat-fastness properties of the dyes are important as to whether an allergic response is caused or not. Polyester dyed with disperse dyes does not in general pose a problem since the sweat-fastness is high. However, problems can arise with polyamide or acetate rayon dyed with disperse dyes, which have a sensitising potential since the low sweat-fastness allows the dyes to migrate to the skin (Wattie, 1987). Indeed, in the 1980s, some severe cases of allergic reactions were reported (Hausen, 1984) relating to stockings made of polyamide and, in the 1990s, to leggings made of acetate rayon (Hausen, 1993). Because of these allergic reactions, the German Federal Institute for Consumer Protection and Veterinary Medicine evaluated the available literature and concluded that the disperse dyes listed in Table 3.2 represent a health risk to consumers and should cease to be used for clothes (Hunger, 2003).

Currently, there is no legal prohibition on these dyes in any country but some organisations, such as the International Association for Research and Testing in the Field of Textile Ecology, which bestows eco-labels on environmentally and toxicologically proven textiles, refuses eco-labels for some dyes (Oko-Tex, 2000).

3.4 Chronic toxicity of textile dyes

Genotoxicity is the major long-term potential health hazard of certain textile dyes. As mentioned in Section 3.2 this became apparent when a high incidence of bladder cancer was observed in plant workers involved in the manufacture

Table 3.2 Disperse dyes considered a health risk to consumers

C.I. name	C.I. no.	CAS no.
Disperse Yellow 3	11855	[2832-40-8]
Disperse Orange 3	11005	[730-40-5]
Disperse Orange 37/76		[12223-33-5]
Disperse Red 1	1110	[2872-52-8]
Disperse Blue 1	64500	[2475-45-8]
Disperse Blue 35		[12222-75-2]
Disperse Blue 106	11935	[68516-81-4]
Disperse Blue 124	111938	[15141-18-1]

of particular dyes during the period 1930–1960. The specific compounds involved (shown in Fig. 3.1) were fuchsine [1], auramine [2], benzidine [3] and 2-naphthylamine [4]. Strict regulations concerning the handling of all known carcinogens have been imposed in most industrial countries, which has caused virtually all dye companies to cease production of these compounds (Hunger, 2003).

Genotoxic chemicals include mutagens, carcinogens and teratogens. Mutagens produce mutations in living organisms. Indeed, one of the first tests involved in screening a new molecule for genotoxicity, the Ames test, assesses whether the chemical causes mutations in the bacterium *Salmonella typhimurium* (Hunger, 2003). Mutagenic chemicals may or may not be carcinogens (cause cancer) in animals and humans. However, since the Ames test is a highly sensitive assay for the induction of point mutations in bacteria, rather than a test for the complex multiple-step process of carcinogenesis in mammals, a close correlation between the Ames test results and rodent cancer assays cannot be expected (ETAD, 1998). Validation studies (Ashby, 1989) show a fairly low degree of correlation between mutagenicity in bacteria and carcinogenicity in rodents. In practice, further tests are carried out in addition to the Ames test. These include further *in vitro* tests, such as the mouse lymphoma test (a gene mutation test) and the cytogenetic test (a chromosome aberration assay). If these tests prove positive, then *in vivo* tests, such as the mouse micronucleus test and the rats' liver unscheduled DNA synthesis (UDS) are done in order to ascertain if the genotoxic potential demonstrated *in vitro* is expressed as cancer in a living rodent.

Teratogens are responsible for birth defects in the offspring of organisms. Thalidomide was a teratogen, causing deformities in babies born in the 1950s. Teratogenicity is very uncommon in textile dyes and is not discussed further.

3.4.1 Effect of physical properties on genotoxicity

Genotoxic chemicals such as mutagens and carcinogens damage DNA (deoxyribonucleic acid), the genetic blueprint material, usually by chemical reaction. Therefore, it follows that any genotoxic chemical must satisfy two criteria:

1. It must reach the DNA (which resides in the nucleus of the cell) in order for the chemical to interact with the DNA.
2. It must possess the ability to interact with the DNA, usually by a chemical reaction.

In order to express a genotoxic effect, a chemical must first come into contact with the DNA present in a cell nucleus. To do this it must be able to transport across the protective cell membranes. Physical factors such as

solubility and molecular size are of paramount importance in determining whether this transport occurs.

In general, smaller molecules are transported across cell membranes more readily than larger molecules. Above a certain molecular size (*c.* MW > 800), molecules become too large to transport across cell membranes. Thus, molecular size offers one way of obtaining non-genotoxic chemicals. Indeed, this approach was adopted by Dynapol to produce non-toxic food dyes (Gordon, 1984). (It is noteworthy that, although the project was technically successful and a small range of prototype polymeric food dyes produced, they never reached the marketplace. Initial tests horrified the volunteers taking part since the dyes were excreted from the body totally unchanged from their original bright colours!). In the textile dye area, phthalocyanine dyes are probably too large to pass through the cell membranes and should be non-genotoxic (Gregory, 1991).

The two extreme cases of high water solubility on the one hand and total insolubility on the other hand generally result in non-genotoxic chemicals (Gregory, 1986; Longstaff, 1983). Pigments, by definition, are insoluble in both water and organic solvents. This insolubility, combined with the relatively large size (*c.* 0.1 to 3 μm) of pigment particles, which are aggregates of millions of individual molecules, ensures that most pigments are not transported across cell membranes. Consequently, the majority of pigments are non-carcinogenic (El Dareer, 1984).

Molecules with high water solubility are also non-genotoxic. There are two major reasons for this. First, the hydrophobic (fatty) nature of the cell membrane is impervious to the hydrophilic water-soluble molecules. Secondly, water-soluble molecules are generally excreted rapidly by a living organism. The best chemical grouping for imparting water solubility is the sulphonic acid ($-SO_3H$) group. Carboxylic acid ($-CO_2H$) groups and hydroxyl (–OH) groups are also useful water-solubilising groups, especially when ionised (Freeman, 2005). These three types of groups are employed extensively in textile dyes. A quaternary nitrogen atom ($-N^+R_4$) also imparts water solubility. This group is found in cationic (basic) dyes.

3.4.2 Classes of carcinogens based on chemical structure

DNA is nucleophilic. Therefore, the active species of most carcinogens, known as the ultimate carcinogen, is an electrophile, E. In most cases, the electrophile is either a nitrenium ion R_2N^+ or a carbonium ion R_3C^+. These ultimate carcinogens attack a nucleophilic site in DNA, which may be a carbon, nitrogen or oxygen atom, to form a covalent chemical bond (equation 3.1).

$$E + [DNA] \rightarrow E\text{–}[DNA] \qquad [3.1]$$

As well as chemical reaction, intercalation is another way for molecules to interact with DNA. In this interaction, a flat portion of the molecule inserts itself into the DNA helix (Gregory, 1991).

3.4.3 Carcinogens based on nitrogen electrophiles

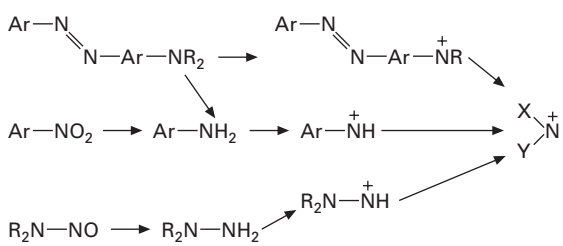
Since an electron-deficient nitrogen atom is a key feature of this class, then obviously all the carcinogens in this class must contain at least one nitrogen atom. The types of chemicals involved vary considerably but include amines, amine derivatives, such as nitrosamines, hydroxylamines and hydrazines, and amine precursors such as nitro compounds. However, the most important type is the amino-containing dye. Figure 3.3 shows how all these compounds produce a common ultimate carcinogen, a nitrenium ion.

Azo dyes are by far the most important class of dye, accounting for over 50% of the world annual production of *c*. 1 million tonnes of dyes (and pigments). Not surprisingly, azo dyes have been studied more than any other class. Therefore, azo dyes will be discussed first.

Azo dyes

The carcinogen may be the dye itself, or it may be a metabolite of the dye. For water-insoluble, but solvent-soluble dyes, such as solvent dyes and disperse dyes, the dye is normally the carcinogen. These dyes usually exist in the azo tautomeric form (Gordon, 1983). For water-soluble dyes, it is a metabolite of the dye which is the carcinogen. These dyes normally exist in the hydrazone tautomeric form. Generally, the azo form has greater stability than the hydrazone form, being more resistant to photo-oxidation (displaying higher light fastness) and to chemical oxidation (displaying better bleach fastness). Indeed, it has been postulated that dyes in the hydrazone form are more easily reduced to their metabolites than dyes in the azo form (Gregory, 1986).

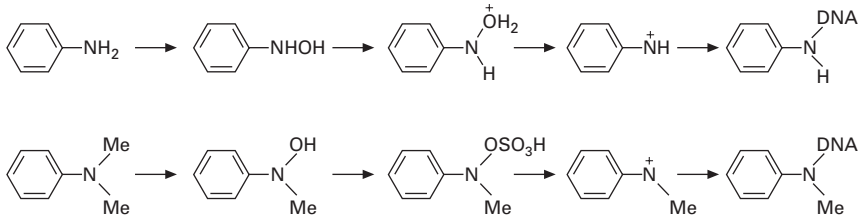
The most prevalent pathway for amine activation for solvent and disperse azo dyes is *N*-hydroxylation. This occurs at a primary or secondary amino



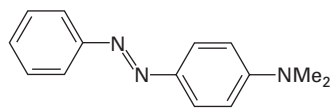
3.3 Carcinogens from nitrogen electrophiles.

group. In dyes containing methylamino- or dimethylamino-groups, the *N*-hydroxylation step is generally preceded by oxidative demethylation. *N*-Hydroxylation appears to be the rate-determining step since it correlates well with the observed carcinogenic activity (Kimura, 1982). Carbon (C– or ring–) hydroxylation can also occur. However, all three oxidative pathways leave the azo group intact (Hunger, 2003), (Hunger, 1994), (Brown, 1993).

The generally accepted mechanism of *N*-hydroxylation is depicted in Fig. 3.4. It applies both to aminoazo dyes, such as Butter Yellow (see Fig. 3.5 [7]), and aromatic amines. Two pathways are shown, one involving a



3.4 Mechanisms for amine activation.



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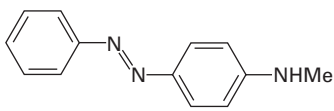

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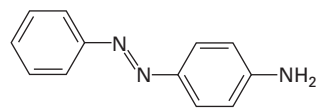

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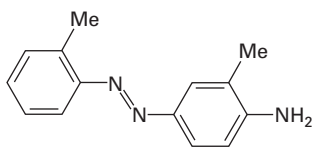

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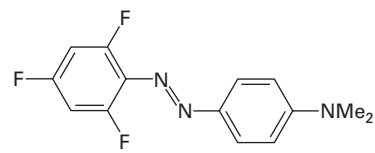

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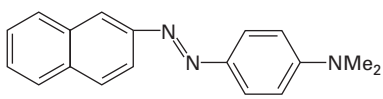

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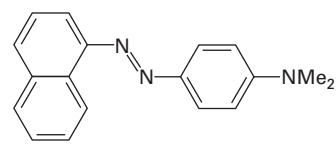
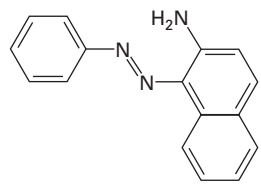


[13]

3.5 Carcinogenic 4-aminoazo dyes including Butter Yellow [7].

dimethylamino-group and one a primary amino-group. In both cases, the *N*-hydroxylated intermediate is formed. The electrophilic species is formed either by acylation of the hydroxyl group (formation of the sulphate ester in the example shown) or by protonation of the hydroxyl group. Both of these are good leaving groups and allow facile reaction with DNA, ostensibly via the nitrenium ion.

The carcinogenicity of aminoazo disperse and solvent dyes has been studied extensively (Beland, 1980; Tarpley, 1980; Kadlubar, 1976; Jen-Kun Lin, 1975a; Jen-Kun Lin, 1975b). Dyes such as Butter Yellow [7] and its analogues may be divided into two groups: 4-aminoazo dyes and 2-aminoazo dyes (Gregory, 1986). A comprehensive study (Longstaff, 1983) showed that all the 4-aminoazo dyes [7–13] in Fig. 3.5 were animal carcinogens. In contrast, the 2-aminoazo dyes [14–16] in Fig. 3.6 were not animal carcinogens. This rather surprising but potentially useful observation may be due to several factors, such as intramolecular hydrogen-bonding, steric hindrance or the facile oxidation to benzotriazole. A plausible mechanism for the reported non-carcinogenicity of 2-aminoarylazo dyes is shown in Fig. 3.7. The nitrenium ion from the 2-aminoarylazo dye is ideally set up for benzotriazole formation. However, not all 2-aminoarylazo dyes are non-carcinogenic (see later).



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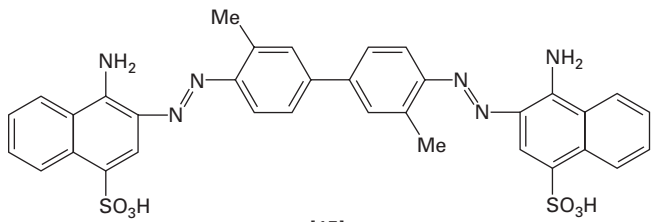

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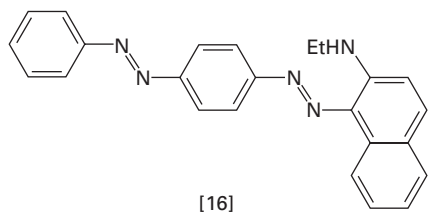

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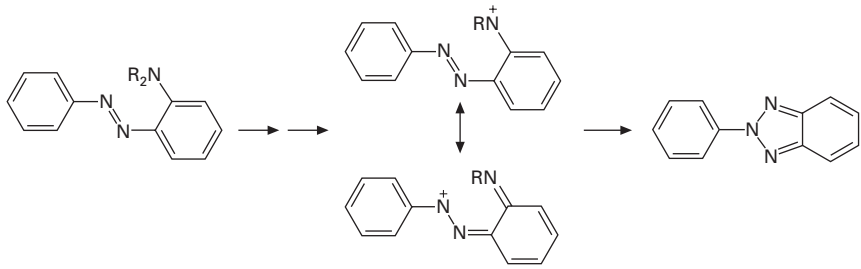
3.6 Non-carcinogenic 2-aminoazo dyes.



3.7 Benzotriazole formation from 2-aminoarylazo dyes.

Dimethylamino-groups and primary amino-groups are implicated in causing mutagenic and carcinogenic effects in aminoazo dyes (Kitao, 1982). However, one way to render such dyes non-mutagenic is to incorporate a cycloalkyl group, such as a piperidino-group, into the dye. Thus, the piperidino-analogue [17], Fig. 3.8, of Butter Yellow [7] is non-mutagenic (Ashby, 1983).

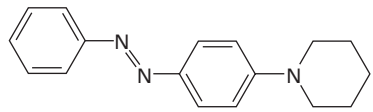
Azophenols also exist in the azo tautomeric form. These dyes are relatively unimportant commercially and have therefore received little attention in terms of toxicology studies. One dye [18], Fig. 3.9, that has been studied was found not to be an animal carcinogen (Gregory, 1986).

Water-soluble azo dyes based on the letter acids such as H-acid, J-acid and Gamma-acid represent a very important class of dyes for dyeing hydrophilic textiles such as cotton and viscose rayon. Cotton is the world's most widely used textile fabric so the tonnages of these water-soluble dyes are extremely large. The dyes are conveniently divided into two types:

1. Those which are capable of generating a carcinogenic metabolite, and
2. Those that are not.

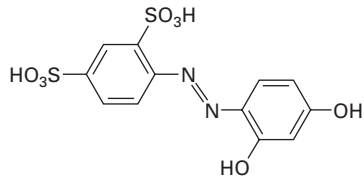
The workers who developed bladder cancer from handling dyes based on benzidine or 2-naphthylamine got the disease not from the dyes themselves, but from the benzidine and 2-naphthylamine metabolites. Indeed, it has been demonstrated that workers exposed to the dust of benzidine-based dyes excreted benzidine and the related metabolites *N*-acetyl and *N*,*N*-diacetylbenzidine (Anliker, 1988). Benzidine has also been detected in the blood serum of female textile workers in dye printing, warehouse and colour room shops (Korosteleva, 1974). The dyes [19–21], shown in Fig. 3.10, are typical of water-soluble azo dyes that generate a carcinogenic metabolite upon reduction in the animal body (Longstaff, 1983), (Gregory, 1986). For example, the dye [21] generates benzidine [3].

There are two main ways to circumvent the carcinogenicity of such dyes. The first way is to use non-carcinogenic analogues of the amines in question, such as benzidine or its derivatives. For example, in Fig. 3.11, C.I. Direct Black 171 [22] uses a non-carcinogenic aromatic benzimidazole diamine [23] (Gregory, 1991) instead of the benzidine [3] used in the similar dye C.I.



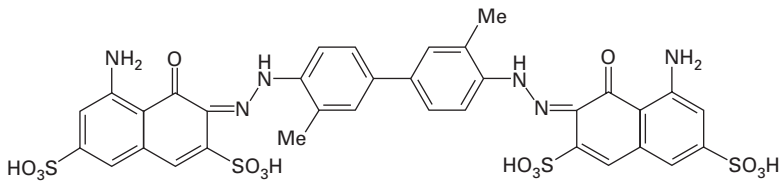
[17]

3.8 The piperidino analogue of Butter Yellow [7].



[18]

3.9 A non-carcinogenic azophenol in the azo tautomeric form.



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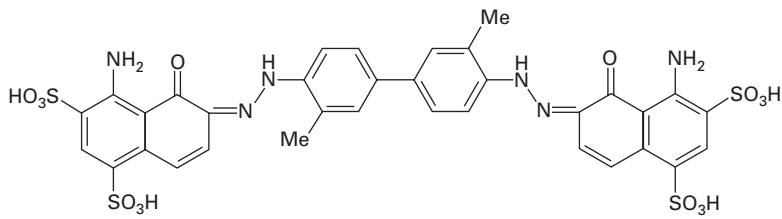

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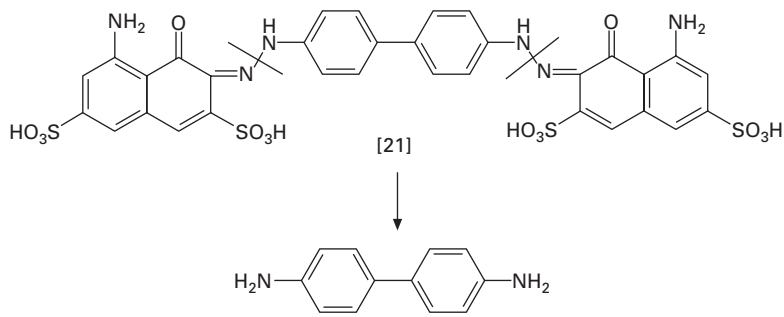
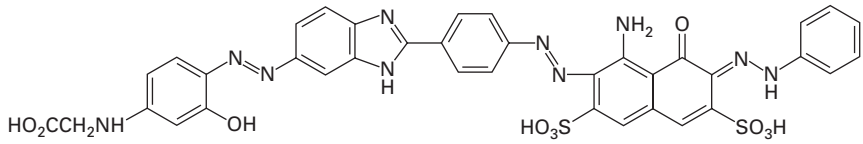


[21]

[3]

3.10 Typical water-soluble azo dyes that generate carcinogenic metabolites.



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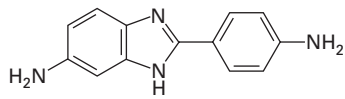
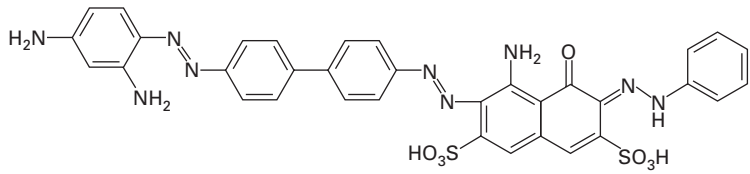


[23]



[24]

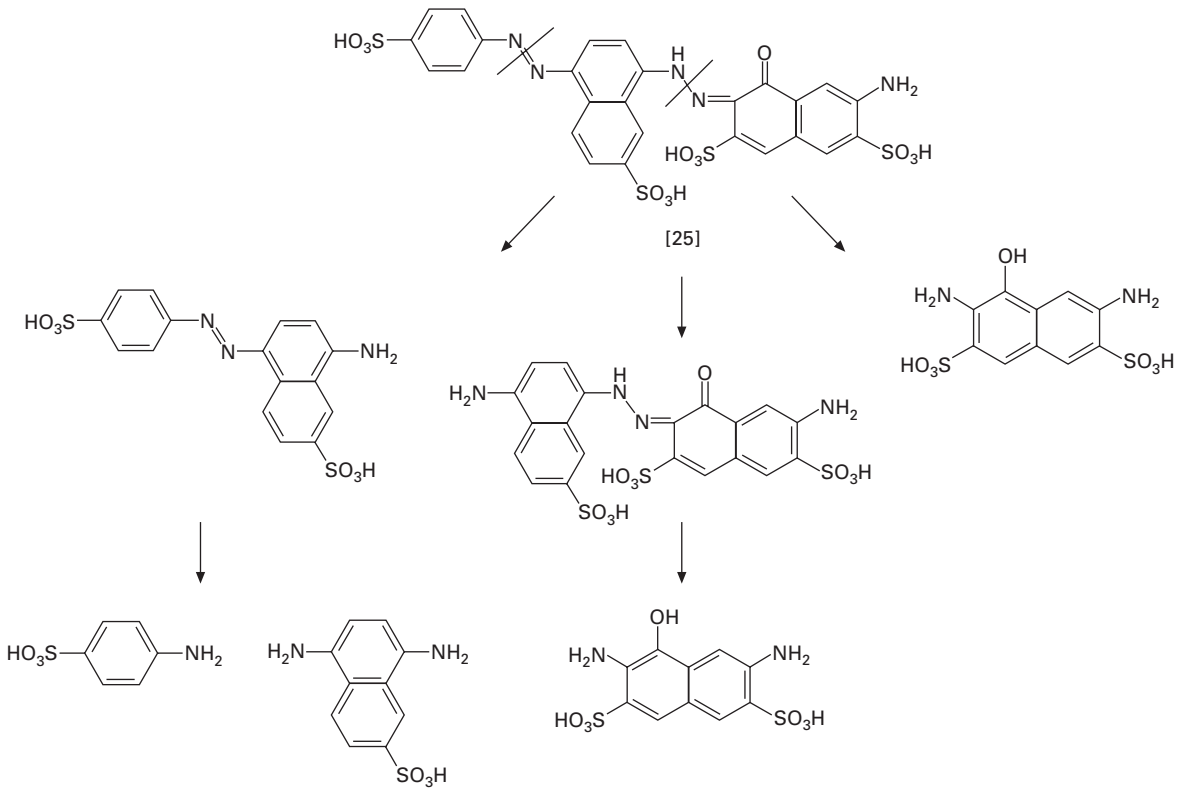
3.11 Use of non-carcinogenic aromatic benzimidazole diamine [23] and benzidine [3] in C.I. Direct Black 171 [22] and C.I. Direct Black 38 [24].

Direct Black 38 [24] (Freeman, 2005). When the latter dye was fed to Rhesus monkeys, benzidine was detected in their urine (Rinde,1975).

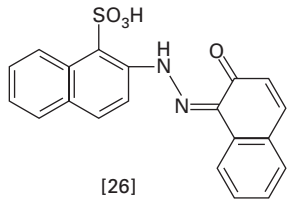
The second way to avoid carcinogenicity is to ensure that all possible metabolites of the dye are water-soluble. An excellent example of this principle is shown in Fig. 3.12, where the degradation of C.I. Food Black 2 [25], a dye used in black inks for ink jet printers (Gregory, 1991), gives metabolites of the dye which contain at least one water-solubilising sulphonic group. This ensures that the dye itself, plus any of its metabolites, are water-soluble.

Further water-soluble dyes that generate water-soluble metabolites and are non-carcinogenic, are given in Gregory (1986). The power of the water-solubilising sulphonic acid group to detoxify dyes and intermediates is beautifully demonstrated by the dye [26] in Fig. 3.13. This dye is non-carcinogenic. Upon reductive cleavage, it would produce, as one metabolite, 2-naphthylamine-1-sulphonic acid (Tobias acid) [27]. As seen earlier, 2-naphthylamine is a potent human bladder carcinogen. However, the presence of just one sulphonic acid group renders it harmless! Indeed, the sulphonic acid group is an excellent detoxifying group both for dyes and their intermediates.

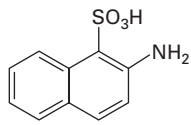
The position of a genotoxic group within a dye also determines whether or not the dye expresses genotoxicity. C.I. Direct Black 17 [28] provides such an example as shown in Fig. 3.14. In this dye, the carcinogen cresidine [29] is present as a middle component (M-component). The dye is a mutagen,



3.12 Probable degradation products of C.I. Food Black 2 [25].

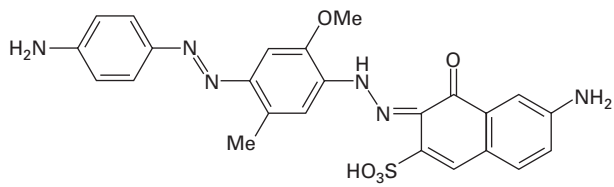


[26]

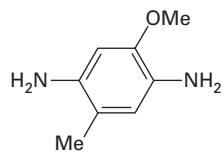


[27]

3.13 Use of a water-solubilising sulphonic acid group in reductive cleavage of dyes to give a non-carcinogenic metabolite.



[28]



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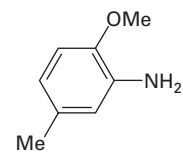
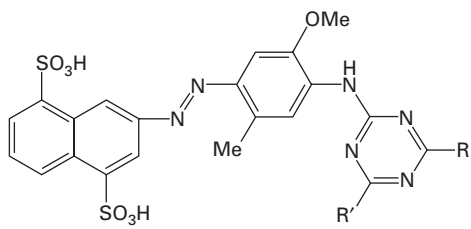


[29]



[31]

3.14 Effect of position of genotoxic group (cresidine [29]) in a dye; [28] is mutagenic but [31] is not.

presumably because of the aminocresidine metabolite [30]. However, the yellow dye [31], in which the cresidine is present as an acylated (triazinylated) end component (E-component), is non-mutagenic. Acylation of the amino-group in cresidine obviously eliminates the mutagenic activity.

Care has to be exercised when using isomers of carcinogens. Thus, 1-naphthylamine is non-carcinogenic. However, during its synthesis, some of the isomeric 2-naphthylamine, a known carcinogen, is produced. This carcinogenic impurity must be removed to a level below which it is not a problem. For dyes that use 1-naphthylamine, every batch must be checked to ensure that the level of 2-naphthylamine is below the recommended level.

In Germany, bladder cancer is recognised as an occupational disease for textile workers (Myslak, 1988). Some dyes have the potential to release an aromatic amine that is known to be a rodent carcinogen upon metabolism in an organism and this has prompted some authorities to conclude that such dyes should be considered to be carcinogenic. This knowledge is the reason for the recommendation of the German MAK Kommission to handle the dyes in the same way as the amines which can be released under reducing conditions. Subsequently, the German, Dutch and Austrian authorities prohibited the use of such dyes in some consumer articles (ETAD 1998). Thus, the dyes may not be used for textile, leather or other articles which have the potential for coming into direct and prolonged contact with human skin, e.g. clothing, bedding, bracelets, baby napkins, towels, wigs (Moll, 1994). The ban, which is across the EU, also covers the import and marketing of the above-mentioned articles dyed with these dyes. Table 3.3 lists the amines that are classified as carcinogenic according to TRGS 614 (Limitation of use of azo dyes which are likely to cleave into carcinogenic aromatic amines (TRGS 614, 2001)) (Hunger, 2003). A list of azo dyes which, upon reduction of the azo group would form the aromatic amines shown in Table 3.3, has been compiled by ETAD. The list includes more than 500 azo dyes, of which at least 142 are still available on the world market (IFOP, 2001).

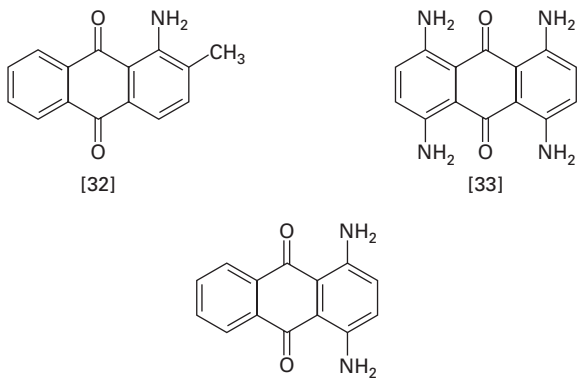
Anthraquinone dyes

From being the second most important class of dye after azo dyes, anthraquinone dyes have declined in importance. Primarily, this is because they have low cost effectiveness due to a combination of low colour strength and relatively expensive manufacture. Consequently, they have been studied less extensively than azo dyes. However, structure–activity relationships in anthraquinone dyes appear to follow a similar trend to those in azo dyes (Brown, 1976). Thus, anthraquinone dyes of the solvent or disperse class containing one or more primary amino- or methylamino-groups tend to be mutagenic or carcinogenic. For example, in Fig. 3.15, C.I. Disperse Orange

Table 3.3 Carcinogenic aromatic amines defined by the German MAK Kommission

C.I. name	CAS no.	Category of carcinogen*
4-Aminobiphenyl	[92-67-1]	1
Benzidine	[92-87-5]	1
4-Chloro-*o*-toluidine	[95-69-2]	1
2-Naphthylamine	[91-59-8]	1
4-Aminoazobenzene	[60-09-3]	2
o-Aminoazotoluene	[97-56-3]	2
4-Amino-3-fluorophenol	[399-95-1]	2
o-Anisidine	[90-04-0]	2
p-Chloroaniline	[106-47-8]	2
4,4′-Diaminodiphenylmethane	[101-77-9]	2
3,3′-Dichlorobenzidine	[91-94-1]	2
3,3′-Dimethoxybenzidine	[119-90-4]	2
3,3′ Dimethylbenzidine	[119-93-7]	2
4,4′-Methylenedi-*o*-toluidine	[838-88-0]	2
4-Methoxy-*m*-phenylenediamine	[615-05-4]	2
6-Methoxy-*m*-toluidine	[120-71-8]	–
4,4′-Methylenebis-(2-chloroaniline)	[101-14-4]	2
4-Methyl-*m*-phenylenediamine	[95-80-7]	2
4,4′-Oxydianiline	[101-80-4]	2
4,4′-Thiodianiline	[139-65-1]	2
o-Toluidine	[95-53-4]	2
2,4,5-Trimethylaniline	[137-17-7]	2
5-Nitro-*o*-toluidine	[99-55-8]	3

*Category 1 denotes a proven human carcinogen, category 2 a proven animal carcinogen and category 3 a suspected animal carcinogen.



3.15 Carcinogenic C.I. Disperse Orange 11 [32] and C.I. Disperse Blue 1 [33] and mutagenic C.I. Disperse Violet 1 [34].

11 [32] and C.I. Disperse Blue 1 [33] are carcinogens (Hunger, 2003), whilst C.I. Disperse Violet 1 [34] is a mutagen (Gregory, 1991).

Some anthraquinone dyes express genotoxicity by intercalation. In this case, they act via insertion of the planar anthraquinone portion of the dye between adjacent base pairs of the DNA helix as shown in Fig. 3.16 (Gregory, 1991).

Cationic dyes

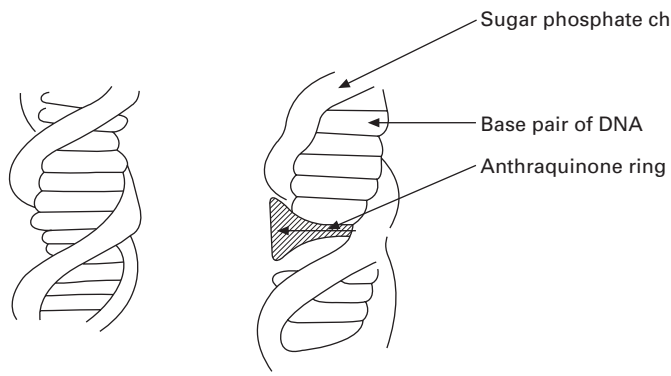
Cationic dyes, along with benzidine and 2-naphthylamine, were implicated in the high incidence of bladder cancer in the textile industry between 1930 and 1960. As seen earlier, the cationic (basic) dyes involved were fuchsine [1] and auramine [2] (Hunger, 2003). Further cationic dyes have been found to be carcinogenic, such as the triphenylmethane dyes C.I. Acid Violet 49 [35] and C.I. Basic Red 9 [36] (Hunger, 2003), and several fluorescent red dyes, such as Pyronine B [37], are mutagenic (Combes, 1982), Fig. 3.17.

Carcinogenic dyes

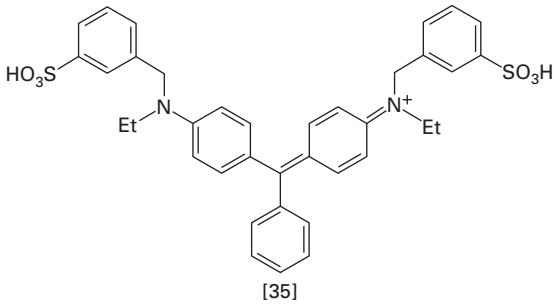
A list of dyes has been compiled that are proven animal carcinogens and which are probably carcinogenic to humans. Table 3.4 lists the dyes that are known to cause cancer in animals and are therefore classified as potential human carcinogens (Hunger, 2003).

Pigments

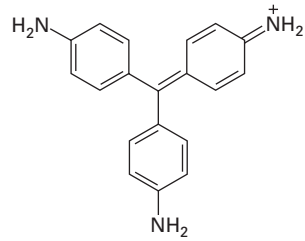
As mentioned earlier, insolubility is an effective way to reduce toxicology. Pigments, by definition, are particulate, insoluble colorants. Therefore, they will be difficult to reduce to the active amine metabolites and extremely



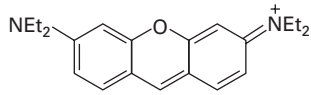
3.16 Intercalation of anthraquinone dyes in DNA.



[35]



[36]



[37]

3.17 Carcinogenic C.I. Acid Violet 49 [35] and C.I. Basic Red 9 [36] and mutagenic Pyronine B [37].

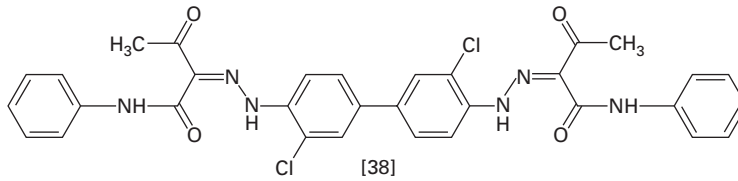
Table 3.4 Dyes classified as potential human carcinogens

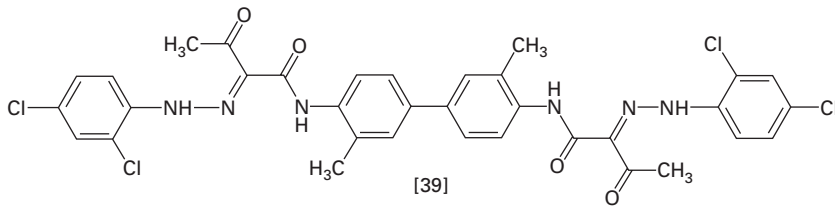
C.I. name	C.I. no	Chemical class
Acid dye	16155	azo
Acid Red 26	16150	azo
Acid Violet 49	42640	triphenylmethane
Basic Yellow 2	42100	ketonimine
Basic Red 9	42500	triphenylmethane
Basic Violet 14	42510	triphenylamine
Disperse Orange 11	60700	anthraquinone
Disperse Blue 1	64500	anthraquinone
Solvent Yellow 1	11000	azo
Solvent Yellow 2	11020	azo
Solvent Yellow 34	41001:1	diphenylmethane

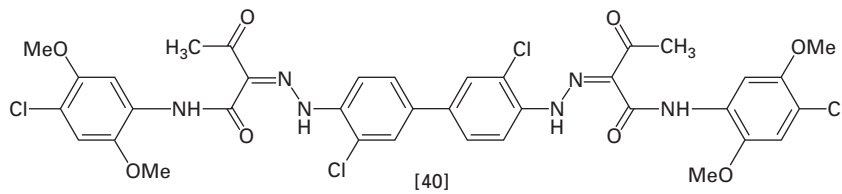
difficult to transport across the cell membranes. Consequently, they should be non-carcinogenic. All three azo pigments, C.I. Pigment Yellow 12 [38], C.I. Pigment Yellow 16 [39] and C.I. Pigment Yellow 83 [40], in the study by Longstaff (1983) were found to be non-carcinogenic (Gregory, 1986), Fig. 3.18.

Aromatic amino- and nitro-compounds

Aromatic amines and aromatic nitro-compounds are particularly important as far as organic colorants are concerned since they are the precursors to many textile dyes (and pigments), especially azo dyes. The most potent carcinogens within this class contain two or more aromatic rings and either primary amino- ($-NH_2$), methylamino- (–NHMe) or dimethylamino- ($-NMe_2$)







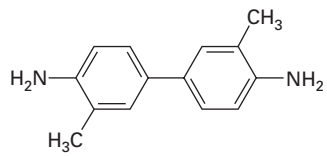
3.18 Non-carcinogenic azo pigments: C.I. Pigment Yellow 12 [38], C.I. Pigment Yellow 16 [39] and C.I. Pigment Yellow 83 [40].

groups. For nitroaromatic compounds, it is believed that a reduced species, such as amino or hydroxylamino, is the carcinogen. Typical compounds include those already discussed, such as benzidine [3] and 2-naphthylamine [4], and the compounds related to benzidine (Fig. 3.19) such as *ortho*-tolidine (3,3′-dimethylbenzidine) [41], *ortho*-dianisidine (3,3′-dimethoxybenzidine) [42], 4-aminobiphenyl [43], 4-nitrobiphenyl [44], 2-aminofluorene [45] and 2-acetylaminofluorene [46] (Gregory, 1991).

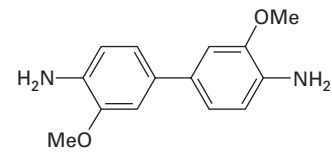
One way to eliminate the carcinogenicity of aromatic amino compounds is to arylate the amine. An excellent example of this principle is provided by dimethyltetraphenylbenzidine [47] Fig. 3.20. In complete contrast to the potent carcinogen benzidine, this compound is non-carcinogenic. It is used widely as a charge transport material in photocopiers and laser printers (Gregory, 1991).

Nitrosamines, hydrazines and hydroxylamines

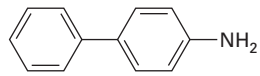
Nitrosamines are of interest because they are formed in every diazotisation reaction of a primary aromatic amine and every commercial azo dye is made by a diazotisation and coupling reaction. However, such nitrosamines pose



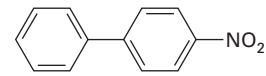
[41]



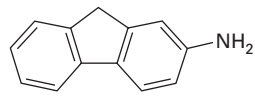
[42]



[43]



[44]



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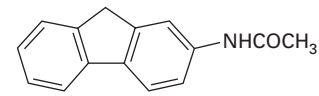
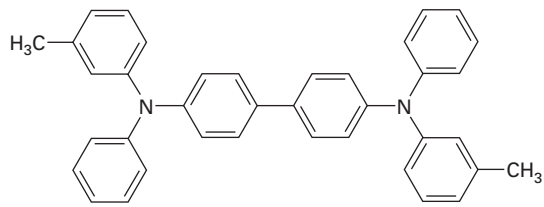


[46]

3.19 Carcinogenic *o*-toluidine (3,3′-dimethylbenzidine) [41], *o*-dianisidine (3,3′-dimethoxybenzidine) [42], 4-aminobiphenyl [43], 4-nitrobiphenyl [44], 2-aminofluorene [45] and 2-acetylaminofluorene [46].



[47]

3.20 Non-carcinogenic dimethyltetraphenylbenzidine [47].

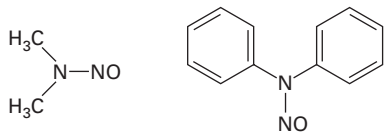
little threat since they are transient species and are contained in reaction vessels. Almost all nitrosamines are carcinogenic, e.g. [48]; the few known exceptions being when the substituents are non-alkyl, such as [49], Fig. 3.21, (Gregory, 1991).

Hydrazine, and many of its derivatives such as dimethylhydrazine [50] and phenylhydrazine [51], Fig. 3.22, are carcinogens; phenylhydrazines are used in the dyestuffs industry to produce heterocyclic coupling components such as pyrazolones.

3.4.4 Carcinogens from carbon electrophiles

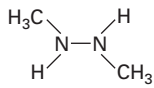
Unlike the carcinogens from nitrogen electrophiles, carcinogens from carbon electrophiles are rarely encountered in dyes. However, they are encountered in the synthesis or chemical modification of dyes.

Carcinogens based on carbon electrophiles may be divided into three types: directly acting alkylating agents, Michael acceptors and polycyclic

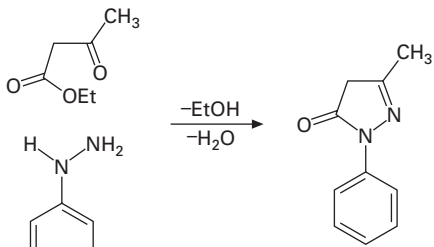


[48] [49]

3.21 Carcinogenic nitrosamine [48] and non-carcinogenic non-alkyl nitrosamine [47].



[50]



[51]

3.22 Dimethylhydrazine [50] and formation of a pyrazolone from phenylhydrazine [51].

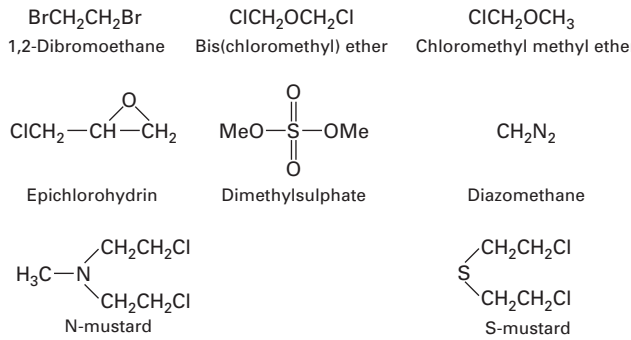
aromatic hydrocarbons. For all three types, the ultimate carcinogen is a carbonium ion.

Direct-acting alkylating agents

Direct-acting alkylating agents contain either alkyl substituents bearing a leaving group, such as chlorine, bromine and methosulphate, or a strained small ring system, usually three or four-membered rings, which ring open to generate the electrophilic centre. Directly acting alkylating agents are employed in the synthesis of the dye (and its intermediates), although chloroalkyl groups have been present in some dyes. Some common examples are shown in Fig. 3.23.

Michael acceptors

Michael acceptors have an ethylene group directly attached to an electron-withdrawing group. Vinyl chloride, acrylamide and acrylonitrile are typical examples.



3.23 Direct-acting alkylating agents: common named examples.

The electrophilic carbon atom is produced by the polarisation induced by the electron-withdrawing group. Like the directly acting alkylating agents, Michael acceptors are used in the synthesis of dyes. For example, acrylonitrile is used to introduce cyanoalkyl groups into disperse dyes as shown in Fig. 3.24. An important exception is the (masked) vinyl sulphone group present in reactive dyes, such as C.I. Reactive Black 5 [52], Fig. 3.25. This important reactive dye was comprehensively studied for its toxicological and ecological profile. It proved to be of low acute toxicity and is non-irritant, a weak sensitiser, and has no genotoxic potential. Also, the hydrolysed dye is not hazardous to effluent water (Hunger, 1991).

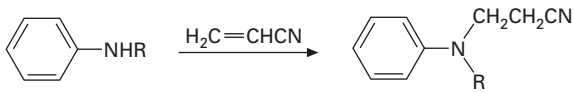
Polycyclic aromatic hydrocarbons

The one-ring, two-ring and three-ring aromatic hydrocarbons benzene, naphthalene and anthracene, respectively, are the basic building blocks for the majority of textile dyes. These lower homologues are usually non-carcinogenic. In contrast, many compounds containing four or more fused benzene rings are carcinogenic. Such compounds are believed to express their activity via epoxide formation, as shown for 1,2-benzanthrene in Fig. 3.26 (Gregory, 1991).

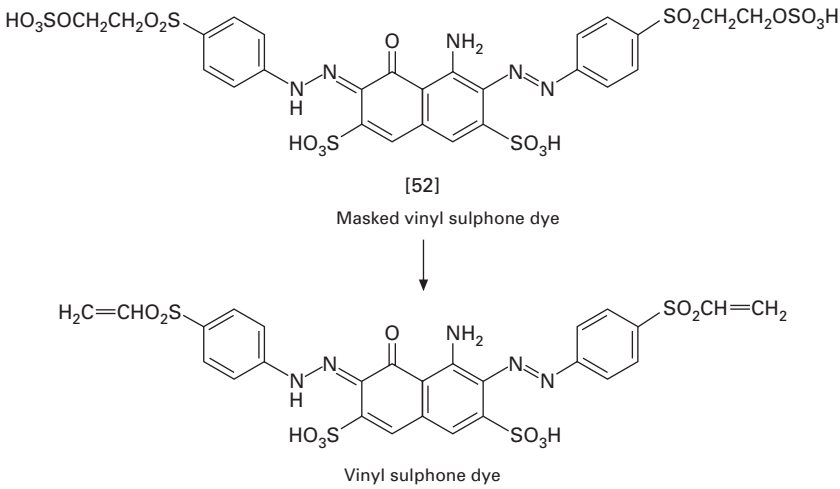
3.4.5 Metals

In water-soluble dyes, the sulphonic acid group is invariably present as a metallic salt, usually sodium, although lithium and potassium are also used. In these dyes, sodium, lithium and potassium pose no problems regarding toxicity. However, heavy metals are also used in dyes and these can present toxicity problems (Stefanovic, 1999).

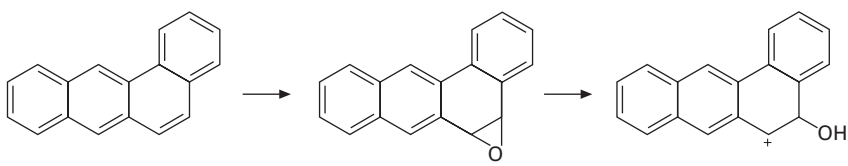
The main source of heavy metals is that from metal–complex dyes. Metal–complex dyes are used for a number of reasons but primarily to improve the



3.24 Cyanoalkylation of an aromatic amine.



3.25 The masked vinylsulphone dye C.I. Reactive Black 5 [52] undergoing hydrolysis.



3.26 Metabolic pathway for polycyclic aromatic hydrocarbons.

light fastness of the dyes. The metals used most frequently in metal–complex dyes are copper (Cu^{2+}), chromium (Cr^{3+}), and cobalt (Co^{3+}), although nickel (Ni^{2+}), is also used to some extent. These metals are chosen since they not only impart the desired properties to the dye, but also they form the most stable complexes. Therefore, under normal use, no free metal should be encountered.

Chromium causes most concern. However, the concern is caused by perception rather than reality. Chromium (VI) (Cr^{6+}), found in chromates, is carcinogenic. Chromium (III), as used in metal–complex dyes, is non-carcinogenic and would not be converted into chromium (VI) under normal conditions. However, they are both chromium.

3.5 Future trends

The production and use of textile dyes is now a very mature industry where the majority of commercial products have been used for many years. As the structure–toxicology relationships of dyes and their intermediates have become better understood, those dyes with known toxicity problems have either been withdrawn or their use strictly regulated. Because of the extensive battery of toxicological (and ecological) testing that a new dye has to pass before it is allowed on to the market, it is extremely unlikely that new dyes will have toxicity problems.

The design of new textile dyes will take advantage of the ever-increasing knowledge of the relationships between the structure of dyes and toxicity (Freeman, 2004, 2005). New dyes will tend to avoid heavy metals, with the important exception of copper phthalocyanine dyes. These dyes produce technically excellent blues and cyans and are the most stable of all the metal–complex dyes.

Efforts will, and indeed are, already being made to reduce toxic components in dyes and produce cleaner, and more environmentally friendly products. For example, Clariant AG has developed a range of sulphur dyes with a much lower sulphide content of 0.3%. This greatly minimises the quantity of the toxic and smelly hydrogen sulphide gas emitted during the dyeing process (Kreutzer, 2004).

The relentless search over the past few decades for better, stronger, more colourful and extremely stable textile dyes, has, inadvertently, caused problems with the disposal of the dyes. The dyes are difficult to degrade in the wastewater treatment plant and some degradation products are toxic (Rossbach, 2000). Indeed, there have been a number of publications recently addressing the treatment of dye effluent and its impact on toxicology (Kandelbauer, 2005; Guivarch, 2004; Upadhyay, 2002; van Lier, 2001; Bahorsky, 1998). In the future, more consideration will have to be given to addressing the balance between the dye properties for its end use and the degradation profile.

Some people have called for a return to natural dyes at the expense of synthetic dyes (Jeet Singh, 2003). Such an approach is seriously flawed. It has been shown (Glover, 1995) that there is not enough arable land in the whole of the world to grow the plants required to generate enough raw material to produce the natural dyes! Nousiainen also asserts there is no way that the annual consumption of 0.5 million tonnes of textile dyes can be met by natural dyes (Nousiainen, 1997). The colours of acceptable natural dyes would be very restrictive, the fastness properties poor, and the costs prohibitive. Also, it is unsound to believe that natural dyes are safe. Of those that have been tested, some have been found to be toxic (Glover, 1995). Indeed, just because something is natural does not automatically mean that it is safe. For

instance, some of the most toxic substances known are natural products, such as aflatoxin B_1, found in peanuts. In the case of peanuts intended for human consumption, the potent animal carcinogen aflatoxin B_1 has to be regulated to parts per billion! (Gregory, 1991). Finally, whilst there is a role for natural dyes to play, it is not a major role.

3.6 Sources of further information and advice

For a general and comprehensive coverage of the toxicology of chemicals, including dyes, the compendium of Sax (Lewis, 1992) is a good starting point. The recent account on the health and safety aspects of industrial dyes (Hunger, 2003), which is referred to several times in the main text, is also highly recommended. The chapter on the toxicology of organic colorants is also useful (Gregory, 1991).

There are several noteworthy reviews worth consulting. These include a survey of azo colorants in Denmark (Ollgard, 1998), Freeman's approach to eliminating toxicity in dyes (Freeman, 2004, 2005), Steingruber's review of the product health impact and toxicology of organic dyes and pigments (Steingruber, 2004), and Desai's and Starodumov's reviews of the toxicology of dyes (Desai, 1992; Starodumov, 1991).

Important specific references are those on the safe handling of dyes (USOC, 1995) and a product stewardship programme for dyes (Helmes, 1994).

ETAD has published numerous Position Papers, Guidelines, lectures and studies on specific problems of colorants concerning toxicology, ecology and legislation. The address is: ETAD General Secretariat, Clarastr.4, CH-4005 Basel, Switzerland, Tel. (+41) 61-690-996, Website www.etad.com. ETAD has also developed an online database containing sources of toxicological, environmental and legal publications on colorants with almost 12 900 documents concerning more than 2000 different dyes (and pigments). It is only available to ETAD member companies.

Material Safety Data Sheets (MSDS) are another useful source of information. They provide the necessary information for safe handling of the dye by the user. Although in Europe they must only be legally provided for hazardous substances according to EU Directive 91/155/EEC, the majority of dye manufacturers provide MSDS for all products, including those that are not hazardous. The Safety Data Sheet contains information such as the identity of the dye, possible hazardous components, and physicochemical, toxicological and ecological data, first aid and emergency measures, occupational exposure limits, and information on personal protective equipment (Sewekov, 1994).

Finally, the research group that is currently most active in studying and designing non-toxic dyes is Freeman's group. The address is: Harold S Freeman, North Carolina State University, Raleigh, USA.

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