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Contact Allergy to Textile Dyes

Clinical and Chemical Studies on Disperse Dyes

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Abstract

Disperse dyes are common sensitizers among textile dyes. Contact allergy to disperse dyes has been documented in studies carried out in southern Europe, however, corresponding studies have not been performed in the Scandinavian countries.

The aim of this study was thus to evaluate the prevalence of allergic reactions to a mix of textile dyes consisting of 8 disperse dyes: Disperse (D) Blue 35, 106 and 124, D Yellow 3, D Orange 1 and 3, and D Red 1 and 17, in southern Sweden, to assess the clinical association between self-reported skin problems and contact allergy to the mix, p-phenylenediamine and related rubber substances, and to ascertain whether patch testing with the dye mix was equivalent to testing with the separate ingredients at the concentrations used in the mix. Furthermore, the purity of the 8 disperse dyes used for patch testing at various dermatology departments and the significance of impurities in preparations of the dyes with regard to contact allergy were studied.

The frequency of contact allergy to the dye mix was 1.5%, which is comparable to the values reported in southern Europe. The most common dye allergen was D Orange 1, while the frequency of test reactions to D Blue 106 and D Blue 124 was lower than expected. The mix was found to be as good a detector of contact allergy to the disperse dyes as testing with any combination of the 8 ingredients tested at the concentrations used in the mix. Contact allergy to p-phenylenediamine was a better indicator of self-reported skin reactions to textiles than the textile dye mix used in this study. Thin-layer chromatography visualized impurities in all 107 investigated patch test preparations. No single reference substance could be identified for D Blue 35. For the other dyes, the mean concentration in preparations labelled 1.0% varied from 0.25%, for D Blue 124, to 0.68%, for D Orange 3. D Orange 3 could not be demonstrated in 4 of 15 preparations labelled D Orange 3. About 25% of the 21 patients diagnosed as being allergic to D Blue 106 and D Blue 124 only reacted to impurities in the patch test preparations when tested with thin-layer chromatograms and a dilution series of commercial and purified D Blue 106 and D Blue 124.

Key words: Contact allergy, Disperse Blue 106, Disperse Blue 124, disperse dyes, Disperse Orange 3, HPLC, impurities, PPD, textile dye mix, textile-related skin problems, TLC.

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Clinical and Chemical Studies on Disperse Dyes

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"And the woman was arrayed in purple and scarlet colour…"

Revelations 17:4

LIST OF PUBLICATIONS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals. The papers are appended at the end of the thesis.

I **Contact allergy to textile dyes in southern Sweden**
Ryberg K, Isaksson M, Gruvberger B, Hindsén M, Zimerson E, Bruze M
Contact Dermatitis 2006: 54: 313-21

II **Is contact allergy to disperse dyes and related substances associated with textile dermatitis?**
Ryberg K, Goossens A, Isaksson M, Gruvberger B, Zimerson E, Nilsson F, Björk J, Hindsén M, Bruze M
British Journal of Dermatology 2009: 160: 107-115

III **Patch testing with a textile dye mix and its constituents in a baseline series**
Ryberg K, Goossens A, Isaksson M, Gruvberger B, Zimerson E, Bruze M
Submitted for publication

IV **Chemical investigations of disperse dyes in patch test preparations**
Ryberg K, Gruvberger B, Zimerson E, Isaksson M, Persson L, Sorensen O, Goossens A, Bruze M
Contact Dermatitis 2007: 58: 199-209

V **Patch testing of patients allergic to Disperse Blue 106 and Disperse Blue 124 with thin-layer chromatograms and purified dyes**
Ryberg K, Goossens A, Isaksson M, Gruvberger B, Zimerson E, Persson L, Bruze M
Accepted for publication in Contact Dermatitis

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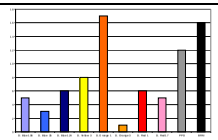
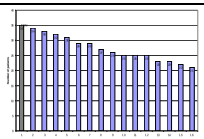
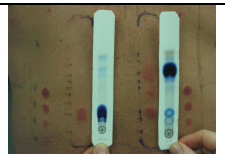
ABBREVIATIONS

ACD	Allergic contact dermatitis
BRM	Black rubber mix
CAS	Chemical Abstract Service
CI	Colour Index
C.I.	Confidence interval
Conc.	Concentration
C.V.	Coefficient of variation
D	Disperse
DB	Disperse Blue
DO	Disperse Orange
DR	Disperse Red
DY	Disperse Yellow
HPLC	High performance liquid chromatography
MS	Mass spectrometry
pet.	Petrolatum
PPD	p-Phenylenediamine
ppm	Parts per million
TDM	Textile dye mix
TLC	Thin-layer chromatography

Thesis at a glance

	Objective	Method	Illustration	Main findings/Conclusions
Paper I **Contact allergy to textile dyes in southern Sweden**	To evaluate the frequency of allergic patch test reactions to a TDM and to the 8 separate dyes in the mix	3325 patients were patch tested with a TDM of 8 disperse dyes. All but 3 of the TDM-positive patients were tested with the separate dyes included in the mix.		The frequency of contact allergy to TDM was 1.5%. The most common dye allergen was DO 1. The high prevalence of allergic reactions to DO 1 was unexpected. The frequency of test reactions to DB 106 and DB 124 was lower than reported in other studies.
Paper II **Is contact allergy to disperse dyes and related substances associated with textile dermatitis?**	To investigate if patient-reported textile-related skin problems could be explained by contact allergy to the 8 disperse dyes, and/or to chemically related substances	A questionnaire on textile-related skin problems was answered by 858 patients before patch testing with the baseline series, including a TDM consisting of 8 disperse dyes and with the separate dyes.		18% of the patients suspected textiles to be the cause of their skin problems. PPD was a better indicator of skin reactions to textiles than the TDM used in this study. Atopic constitution and female gender were found to be risk factors for skin reactions.
Paper III **Patch testing with a textile dye mix and its constituents in a baseline series**	To investigate if patch testing with a TDM consisting of 8 disperse dyes was equivalent to testing with the separate ingredients at the same concentrations as in the mix	1780 patients were tested with a TDM consisting of 8 dyes, with the separate ingredients at the same concentrations as those in the mix, and also with the 8 dyes at a concentration of 1.0%.		The TDM was as good a detector of contact allergy to the disperse dyes as testing with any combination of the ingredients at the concentration used in the mix.
Paper IV **Chemical investigations of disperse dyes in patch test preparations**	To investigate 8 disperse dyes used for patch testing at the departments in Malmö and in Leuven and to compare them with test preparations used at other dermatology departments	107 patch test preparations from 13 clinics were analysed, using HPLC and TLC, and compared with reference substances obtained at the Malmö department.		The mean concentration in the preparations was lower than the stated concentration for all dyes. Variations were found between the samples with regard to the number of impurities. DO 3 could not be demonstrated in 4/15 preparations labelled DO 3.
Paper V **Patch testing of patients allergic to Disperse Blue 106 and Disperse Blue 124 with thin-layer chromatograms and purified dyes**	To investigate the significance of impurities in preparations of disperse dyes containing DB 106 and DB 124 with regard to contact allergy	21 patients allergic to DB 106 and/or DB 124 were tested with dilution series of commercial and purified DB 106 and DB 124 and with TLC strips made from preparations containing DB 106 and DB 124.		Approximately 25% of the patients diagnosed as being contact allergic to DB 106 and DB 124 only reacted to impurities in the patch test preparations.

DB, Disperse Blue; DO, Disperse Orange; HPLC, high performance liquid chromatography; PPD, p-phenylenediamine; TLC, thin-layer chromatography; TDM, textile dye mix.

CONTENTS

1. INTRODUCTION 3
1.1 Background 3
1.2 Textile dyes 4
1.2.1 Chemical properties 5
1.2.2 Classification of dyes according to application 6
1.2.3 Classification according to chemical structure 9
1.2.4 Environmental aspects 9
1.2.5 Adverse effects 10
1.2.6 Regulations 11
1.3 Disperse dyes 14
1.3.1 Properties and use 14
1.3.2 Contact allergy and allergic contact dermatitis 15
1.3.3 Contact allergy and allergic contact dermatitis from disperse dyes 16
1.3.4 Sensitizing potential 17
1.3.5 The prevalence of contact allergy 18
1.3.6 Skin manifestations 20
1.3.7 Patch testing 20
2. AIMS 22
3. MATERIALS AND METHODS 23
3.1 Subjects 23
3.2 Chemicals and patch test preparations 24
3.3 Patch testing 27
3.3.1 Patch testing with thin-layer chromatograms 28
3.3.2 Patch test technique 28
3.3.3 Evaluation of patch tests 29
3.4 Questionnaire 29
3.5 Chemical investigations 30
3.5.1 Reference substances 30
3.5.2 Preparations of samples from patch test preparations 30
3.5.3 High performance liquid chromatography 31
3.5.4 Thin-layer chromatography 31
3.6 Recording of reactions 32
3.7 Ethics 32
3.8 Statistical calculations 32

4. RESULTS 33
4.1 Patch testing 33
4.1.1 Patch testing with the disperse dyes at different concentrations 35
4.1.2 Degree of hypersensitivity 36
4.2 Testing with thin-layer chromatograms 36
4.3 Questionnaire on skin problems arising from textiles 40
4.4 Chemical investigations 44
4.4.1 High performance liquid chromatography 44
4.4.2 Thin-layer chromatography 45
5. DISCUSSION 51
5.1 Patch testing with a textile dye mix in the baseline series 51
5.2 Patch testing with the ingredients in the textile dye mix 52
5.2.1 Cross-reactivity 56
5.3 Clinical relevance of a positive patch test 57
5.3.1 Nature of patch test reactions 57
5.3.2 Exposure 58
5.4 Differences in patch test results Leuven vs. Malmö 60
5.5 Gender differences in patch test results 60
5.6 Chemical investigations 61
5.6.1 Concentration of purified dyes and impurities 61
5.6.2 Incorrect labelling 62
6. SUMMARY AND CONCLUDING REMARKS 65
SUMMARY IN SWEDISH 67
ACKNOWLEDGEMENTS 71
REFERENCES 73

1. INTRODUCTION

The aim of the work presented in this thesis was to investigate clinical and chemical aspects of contact allergy to disperse dyes. A general introduction to textiles and textile dyes is given here to provide the reader with an understanding of the topic.

1.1 Background

Clothes help to protect the human body from sunlight, heat and cold. They are worn for safety, comfort and modesty, and reflect religious, cultural and social values. Clothing offers protection against many things that can injure the naked body, but some protective clothing, such as that worn by hospital staff, may also protect the environment from the wearer. The distinction between clothing and protective equipment is not always clear; consider, for example, diving suits, swimsuits, bee-keeper's clothing and motorcycle leathers.

Clothing and other textiles can be made from natural fibres such as cellulose (cotton, linen), or from protein (wool, silk). Synthetic fibres may consist of cellulose derivates (rayon, viscose, acetate and triacetate), polyamides (nylon, with the brand names Antron, Perlon, etc.), polyesters (Dacron, Terylene), acrylics (Acrylan, Courtelle, Dralon), or fibres with elastic properties, so-called elastomers (elastane, Lycra, Spandex). Furthermore, fibres are often blended (1,2). Textile fibres themselves are usually not allergenic, but they may be responsible for irritant contact dermatitis (3,4). However, silk has been reported to give both immediate and delayed hypersensitivity (5). Allergic contact dermatitis (ACD) arising from nylon is uncommon, but can in exceptional cases be caused by monomers used in nylon synthesis. Patients with an atopic constitution and/or a sensitive skin often complain of intolerance to clothes, especially woollen garments (6) and synthetic fibres. Textile finish resins, used for cotton, cotton/polyester and linen garments to provide crease resistance, have been used since the 1920s, and have been reported to cause contact allergy (7-9). Most textile finish resins are thought to release formaldehyde. As formaldehyde is a contact allergen (10), patients who are already allergic to formaldehyde often report textile-related skin problems. In the past 20 years, however, the textile industry has reduced the level of formaldehyde residues in clothing, and most clothing today does not contain enough free formaldehyde to cause skin problems in formaldehyde-allergic individuals (11-13).

Textiles may contain hazardous chemicals, for example, perfluorinated compounds (14) used in water- and dirt-repellent textiles. Furthermore, bactericides such as triclosan were previously used in sportswear, underwear, socks, and cycling trousers, but their use has decreased in favour of silver compounds and silver threads (15).

Several reports on the environmental effects of these chemicals have been published in recent years (15-17). However, the influence of these substances on humans and the true prevalence of skin manifestations resulting from substances in clothing and other textiles are not known (8,18).

1.2 Textile dyes

Colour has always been important to humans. According to archaeological records, early humans, particularly in India and the Middle East, developed methods of dying fibres and used them to decorate fabric for clothing, household, and ceremonial items. Coloured textiles have been found in 5000-year-old Egyptian tombs. The dyes were of animal, vegetable or mineral origin, with no or very little processing (19). Dyeing textiles was a precursor to embroidery. Dyes giving bright, permanent colours were difficult to find and therefore such dyes and the items dyed with them were valuable commodities.

The first synthetic dye was discovered by the English chemist William Henry Perkin in 1856 at the age of 18 (19,20). While searching for a cure for malaria by oxidizing aniline he found mauveine, a basic dye, also known as aniline purple and Perkin's mauve (Figure 1). It had a brilliant colour, but faded easily. This was the start of the rapid development of new synthetic dyes, and these dyes quickly replaced traditional natural dyes. They cost less, they offered a wide range of colours, and they had better dyeing properties. Today, there are thousands of textile dyes belonging to different classes, many of them marketed under different trade names. The final colour of a textile is often the result of a mixture of dyes (21). Furthermore, many dyes often contain not only the main component, but also several impurities (22,23).

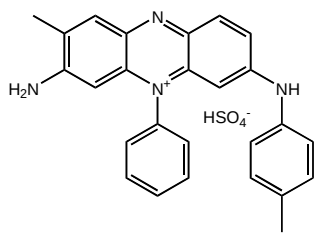


Figure 1. *Mauveine A, one of 4 related aromatic compounds in the mixture called mauveine, discovered by William Perkin in 1856.*

Possible contact allergic reactions to synthetic textile dyes have been recognized since 1868 (20,24). In 1884, the influence of new synthetic dyes was debated in *The Times* and in medical journals in England, as a result of the numerous complaints of unacceptable skin eruptions, "caused by wearing, when in the state of perspiration,

hosiery and flannel coloured with aniline dyes" (19). In 1940, nylon stockings were introduced into the American market. Soon after their introduction, cases of nylon stocking dermatitis were reported. This was originally called "nylon allergy". Upon further investigation, however, it was found that the dermatitis was caused by dyes and not by nylon (3,25). In 1947, Dobkevitch et al. reported eczema due to cross-hypersensitivity between the azo dye Disperse Yellow (DY) 3 in nylon stockings and the para-amino compound p-phenylenediamine (PPD), today mostly used as a hair dye (26). Starting in 1985, several authors reported patients with ACD caused by dark clothes, mainly due to contact allergy to other disperse dyes such as Disperse Blue (DB) 106 and DB 124 (21,27,28).

1.2.1 Chemical properties

A dye absorbs a particular part of visual light, thus appearing to have a specific colour. The chemical groups present in the dye molecule decide its colour. Dye molecules must contain a system of conjugated double bonds, called the chromophore, which is linked to electron-donating groups (auxochromes, $–NH_2$, –OH, etc.) and electron-accepting groups (anti-auxochromes, $–NO_2$, >C=O, etc.). The assembly of a chromophore, an auxochrome and an anti-auxochrome, is sometimes called a chromogen (29).

Azo and anthraquinone dyes are the 2 most important classes used for dyeing textile fibres today (Figure 2). More than 50% of dyestuff production is in the azo dye group (30). They are used in various products such as hair dyes, foods (31), tattoo dyes (32), and colouring agents for drugs (33), paper and textiles (2). Azo dyes are found in several groups of textile dyes, i.e. disperse, reactive and acid (see Section 1.2.2), and they are used for dyeing cotton and linen, and synthetic fibres such as polyester, acetate and nylon (34). Azo dyes are characterized by the presence of at least 1 azo group (-N=N-) in the chromophore (35). Anthraquinone dyes have 2 carbonyl groups (>C=O) in the chromophore.

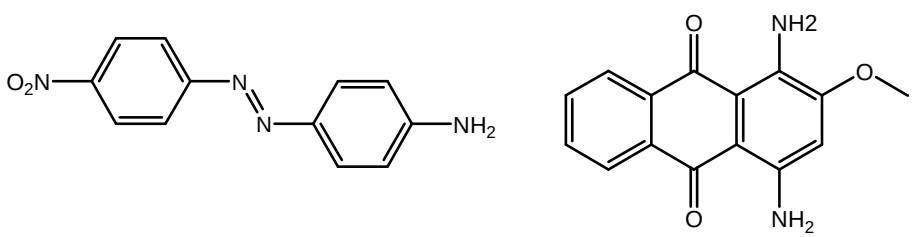


Figure 2. *Examples of an azo dye (Disperse Orange 3) on the left and an anthraquinone (Disperse Red 11) on the right.*

1.2.2 Classification of dyes according to application

The classification of textile dyes according to their application to different fibres can be found in Table 1.

Table 1. *Classification of textile dyes according to application (compiled from a table published by Hatch et al. (36)).*

Fibre group based on origin and polymer chemistry	Class name	Acid (anionic)	Basic (cationic)	Disperse	Direct	Reactive	Azoic/vat/ sulphur	Mordant	Pigment
Natural fibres composed of cellulose polymers	Cotton				x	x	x	x	x
	Flax (linen)				x	x	x		
Natural fibres composed of protein (animal fibres)	Wool	x	(x)		x	x	(x)	x	x
	Silk	x	(x)		x	x	(x)		x
Man-made fibres from chemically modified cellulose	Rayon/viscose				x	x	x		x
	Acetate			x					x
	Triacetate			x					
Man-made fibres composed of synthetic polymers	Nylon/polyamide	x	x mod.	x mod.	x	x			x
	Polyester		x mod.	x					x
	Acrylic polyacrylonitrile	x mod.	x	(x)					x
	Polypropylene			x					
Man-made synthetic stretch fibres	Spandex/elastane	x mod.		x	x mod.				x

x mod., a modified form of the fibre can be dyed with this dye.

Acid dyes are water-soluble anionic dyes, characterized by the fact that the dye contains one or more acid groups. They have a rather low molecular weight and are usually available in the form of a salt, such as the sodium salt of the sulphonic acid (34). They are resistant to sunlight and washing. Acid dyes may be applied to fibres such as silk, wool, nylon and modified acrylic fibres, but they do not bind to cellulose fibres. They can also be used for dyeing leather and cosmetics. Binding to the textile fibre is attributed to salt formation between the anionic groups in the dyes and cationic groups in the fibre. Acid dyes include both azo and anthraquinone compounds (2,34). Contact allergy to acid dyes has been documented, and some acid dyes, such as Acid Yellow 61, Acid Red 118 and Acid Red 359, are included in commercially available textile patch test series. Allergic reactions to acid dyes in surgical suture material have also been reported (37,38).

Azoic (or naphthol) dyes are water-insoluble azo dyes used for dyeing cellulose fibres, mainly cotton. Azoic dyes are produced directly on or within the fibre by applying two chemically reactive water-soluble compounds to the fabric. This is achieved by treating the fibre with a diazoic compound and a coupling component, 2-hydroxy-3-naphthanilide (Naphthol AS), which has an affinity to cellulose. With

suitable adjustment of the dye-bath conditions, the two components react to produce the insoluble azo dye. This technique of dyeing is unique, in that the final colour is controlled by the choice of diazoic and coupling components. Some diazo compounds are considered to have carcinogenic properties, and Naphthol AS has been reported to be a sensitizer in both industrial workers (39) and consumers, with hyperpigmentation being a typical manifestation of contact allergy (40-42). Naphthol AS is included in some textile patch test series.

Basic dyes are also called cationic dyes, as they contain positively charged groups. Basic dyes are salts of amines, soluble in water and alcohol, and are mainly applied to acrylic fibres, but are sometimes used for dyeing wool, silk and paper (34). They can be applied to cotton with a so-called mordant (a substance used to fix dyes in fabrics by forming an insoluble compound with the dye). These dyes are often brilliant, but have poor resistance to light. Contact allergy to basic dyes has been reported, for example, to Basic Black 1 and Basic Brown 1 (43), as well as to Basic Red 22 in hair-colouring mousse (44) and Basic Red 46 in acrylic blend socks (45).

Direct dyes, also called substantive dyes, are applied directly to the fibres. They are used in a neutral or slightly alkaline dye bath, with the addition of either sodium chloride or sodium sulphate. Direct dyes are used on cotton, wool, silk, nylon, viscose, leather and paper. They have low wet-fastness, and often require after-treatment by the addition of cationic fixatives (2,34). The affinity to the fibres can also be increased by enlarging the molecule. This is done by introducing more azo groups, resulting in di-, tri- and polyazo dyes (29). Direct Orange 34, which has been reported to cause contact allergy (46), is included in some textile patch test series.

Disperse dyes were originally developed for the dyeing of cellulose acetate and are partially soluble in water. Their main use nowadays is in dyeing polyester, but they can also be used to dye nylon, cellulose triacetate and acrylic fibres (2). (See Section 1.3).

Mordant dyes are also called metal complex dyes. They are acid dyes, with hydroxyl or carboxyl groups in the molecule (Figure 3). They are mainly used for dyeing wool and silk. These dyes form chelates or organometallic complexes with metal ions such as chromium, aluminium, copper, iron, tin and cobalt (34). Most natural dyes belong to this class, but the most important are the synthetic mordant dyes, the chrome dyes, used for wool. In 1978 Fregert et al. described two patients, previously sensitized to chromium, who exhibited ACD resulting from green military uniforms. Chemical analysis showed that water-soluble chromium(III) was released from the uniforms even after repeated washing (47).

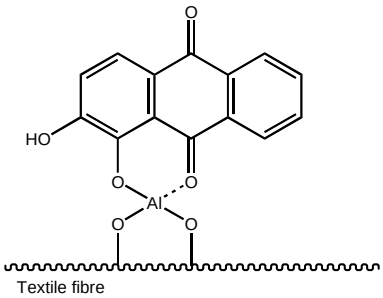


Figure 3. *A schematic illustration of Mordant Red 11 forming a chelate complex with aluminium on a textile fibre.*

Natural dyes are of mineral, animal or plant origin. They often have a complex composition and many of them lack a chemical definition. However, some of them belong to the acid and vat dyes (see below). They are only rarely used today, with the exception of hobby activities (34). Indigo was initially a natural dye, extracted from several species of plants, but today nearly all indigo is synthetically produced (see vat dyes) (48).

Reactive dyes bind to natural fibres by covalent bonds, making them among the most permanent dyes with a high wet-fastness. They are used for dyeing cotton and other cellulose fibres, protein fibres and nylon (34). Eighty percent of the reactive dyes have an azo structure (29). Reactive dyes have been reported to cause both type I mediated allergic reactions such as asthma, rhinitis and urticaria, and type IV hypersensitivity reactions (49-51), mostly following occupational exposure.

Sulphur dyes have a high molecular weight and are often insoluble in water, but are soluble in a sodium sulphide solution. These dyes are complex and their structure is not fully understood (29). Sulphur dyes are cheap, have good washing-fastness and are easily applied. They are used for dyeing cotton and linen, e.g. dark working clothes (2).

Vat dyes are insoluble in water and incapable of dyeing fibres directly. However, reduction in an alkaline liquor produces the water-soluble alkali metal salt of the dye, which has an affinity for textile fibres. Subsequent oxidation reforms the original insoluble dye (20). Vat dyes are used especially for dyeing cotton, linen, wool and viscose (34). Vat dyes include derivates of anthraquinones, and batik dyes also belong to this group. Synthetically produced indigo is used for dyeing denim.

Solvent dyes are mono- or diazoic compounds used to dye organic solvents such as various kinds of oils, waxes, lubricants, plastics and fuels. Fibres that are difficult to dye in aqueous solutions may be dyed with solvent dyes (34), but as they are added

during the production of synthetic fibres they are not regarded as true textile dyes. Contact allergy to Solvent Yellow 1 (p-aminoazobenzene) has been reported in patients reacting to stockings (52), and to Solvent Red 179 in patients reacting to the earpieces of spectacle frames (53). Allergic reactions to Solvent Yellow 2 (p-dimethylaminoazobenzene), Solvent Yellow 14 and Solvent Orange 8 (54) have also been documented.

Pigments are mostly mono- or diazoic compounds, insoluble in the vehicle used. Pigments are used for colouring paint, ink, plastic, fabrics, cosmetics, food and other materials, for textile printing and for colouring synthetic materials before the production of textile fibres. The pigment must be bound to the surface of the material by means of a binding agent (34).

1.2.3 Classification according to chemical structure

Information about textile dyes can be found in the Colour Index (CI) (55). Each dye has a CI generic name composed of the application class, the colour and a number, e.g. Disperse Orange (DO) 1. Furthermore, dyes with a defined structure have a CI number; for example, DO 1 has the CI number 11080. The CI also contains structure images, uses, commercial names and producers of the dyes. The Chemical Abstracts Service (CAS), a division of the American Chemical Society (56), also assigns identifiers to chemicals that have been described in the literature by giving them a CAS number, which is a unique numerical identifier. A dye may have a defined CAS number, but no CI number if the chemical formula of the dye is not known.

1.2.4 Environmental aspects

Environmental problems from the dyeing of textiles arose after industrialization, when traditional natural dyes were replaced by synthetic dyes (19). Since synthetic dyes are designed to resist chemicals, and thus improve the quality of the product, they are also persistent in the environment. Some dyes can be transformed into carcinogenic compounds in the environment. The release of untreated wastewater from the dyeing industry is a threat to the environment. Furthermore, the discharge of coloured effluents into watercourses affects the penetration of sunlight, which in turn decreases the level of photosynthetic activity and subsequently the dissolved oxygen level. It is difficult to find information on the environmental impact of dyes, as only limited information is available on a small number of substances in the standard toxicological and environmental literature. However, results of studies on the biodegradability of dyes in aqueous environments have shown that most of the organic dyes are not readily degradable, and there is reason to suspect that all organic dyes are resistant to degradation, unless proven otherwise (34). Furthermore, the

polluting nature of dyes is an ecological problem as many dyes, especially disperse dyes, are fat-soluble substances that may be accumulated in fish and in other aquatic organisms (29). Since the majority of dyes are resistant to conventional biodegradation the most common degradation techniques in use today are physical-chemical ones (57). However, promising methods employing sequential anaerobic-aerobic treatment have been described (58). A similar project employing biological treatment methods using fungi and bacterial degradation of dyes with anaerobic-aerobic treatment, as well as photocatalytic degradation of synthetic textile dye wastewater is in progress at the Department of Biotechnology, Lund University, Sweden (Maria Jonstrup, personal communication, 2009).

In 1974, the Ecological and Toxicological Association of Dyes and Organic Pigments (ETAD), an association of dye manufacturers, was formed to represent the interests of the industry. The ETAD is now an international organization with member companies in 16 countries and operating committees in different parts of the world. The aim of the ETAD is to minimize possible negative effects on health and the environment arising from the manufacture and use of synthetic organic colourants, and to ensure that information on practicable protection is provided to the purchasers of dye products (59).

1.2.5 Adverse effects

Azo dyes constitute the largest chemical class of synthetic textile dyes because of their versatility, low price, and ease of production. These dyes may, however, pose a health risk to humans due to the formation of mutagenic/carcinogenic aromatic amines upon reductive cleavage of their azo groups (-N=N-) (60). The best known aromatic amine is aniline, but another example is the potent human bladder carcinogen benzidine, which is derived from the reduction of several azo dyes (61,62). Bacteria isolated from healthy human skin and human skin bacteria from strain collections have shown azo reductase activity under *in vitro* conditions. This means that the cleavage of azo dyes by the skin microflora, and the formation of aromatic amines, may be possible (63). For example, it has been suggested that DO 3 is degraded into PPD and nitroaniline in the skin (2,62). On the other hand, the results of some studies indicate that anaerobic conditions, present only in the intestines, are required for the degradation of azo dyes (61). More studies must be carried out to determine the pathways of degradation of dyes and the metabolic/toxic properties of their degradation products.

Systemically ingested azo dyes used in food, sweets, soft drinks and as colouring agents for drugs have been blamed for provoking immediate type hypersensitive reactions such as urticaria and asthma bronchiale (64). Clinical studies indicate that one azo dye, tartrazine (E 102), is occasionally associated with flares of urticaria or

asthma. The mechanism of sensitivity to tartrazine is obscure, and has been called "pseudoallergic" (31). Additional studies must be conducted to clarify the cause and the mechanisms involved (65). There is no convincing evidence in the literature of reactivity to other systemically ingested azo dyes (66). However, de Leysat et al. reported two patients with contact allergy to PPD, presenting with exacerbated eczema following the ingestion of sulphanilic acid, a metabolite of Sunset Yellow (E 110), an azo dye. The authors speculated that the worsening cutaneous condition of their patients could be attributed to cross-sensitivity between PPD and sulphanilic acid (4-aminobenzenesulphonic acid) (33).

1.2.6 Regulations

Within the EU, azo dyes that can release carcinogenic aromatic amines at a concentration exceeding 30 ppm in the finished product or in the dyed parts thereof are forbidden in accordance with EU Commission Directive 76/769/EEC, and the latest amendment from January 2003 (2003/3/EC) (Table 2) (67). From June 1, 2009 the regulations will follow Regulation 1907/2006 (Reach) Annex XVII (68). Azo dyes with the aforementioned properties may not be used in textile and leather articles which may come into direct and prolonged contact with the human skin or oral cavity, such as: clothing, bedding, nappies and other sanitary items, footwear, gloves, chair covers, textile or leather toys and fabrics intended for use by the final consumer.

According to another EU decision from May 15, 2002, which established the ecological criteria for the EU eco-labelling of textile products, the producer must either provide a declaration of non-use of the disperse dyes listed in Table 3 as carcinogenic or allergenic, or provide a test report proving their colour fastness (69).

The Oeko-Tex Association, a group of 14 textile research and test institutes in Europe and Japan, has also listed carcinogenic and allergenic dyes, the use of which is forbidden in clothes certified and marked with the Oeko-Tex label (Table 3) (70). Many large clothing chains such as H&M follow the recommendation of Oeko-Tex, not to use these dyes in their clothes production. However, EU regulations are only binding for EU countries, and do not have to be observed in Asian countries or in the Eastern European markets outside the European Union (62).

Table 2. *Compilation of aromatic amines, including Chemical Abstract Service (CAS) numbers, listed by the EU Commission as carcinogenic.*

Substance	CAS number
4-aminobiphenyl	92-67-1
benzidine	92-87-5
4-chloro-o-toluidine	95-69-2
2-naphthylamine	91-59-8
o-aminoazotoluene	97-56-3
2-amino-4-nitrotoluene	99-55-8
p-chloroaniline	106-47-8
2,4-diaminoanisole	615-05-4
4,4'-diaminodiphenylmethane	101-77-9
3,3'-dichlorobenzidine	91-94-1
3,3'-dimethoxybenzidine	119-90-4
3,3'-dimethylbenzidine	119-93-7
3,3'-dimethyl-4,4'-diaminodiphenylmethane	838-88-0
p-cresidine	120-71-8
4,4'-methylene-bis-(2-chloro-aniline)	101-14-4
4,4'-oxydianiline	101-80-4
4,4'-thiodianiline	139-65-1
o-toluidine	95-53-4
2,4-diaminotoluene	95-80-7
2,4,5-trimethylaniline	137-17-7
o-anisidine (2-methoxyaniline)	90-04-0
4-aminoazobenzene	60-09-3

Table 3. *Compilation of individual dyestuffs, including Chemical Abstract Service (CAS) and Colour Index (CI) numbers, listed by the EU Commission and by Oeko-Tex as having carcinogenic or allergenic properties.*

Dyestuffs classified as being carcinogenic		
Generic Name	CI number	CAS number
Acid Red 26	16 150	3761-53-3
Basic Red 9	42 500	569-61-9
Basic Violet 14	42 510	632-99-5
Direct Black 38	30 235	1937-37-7
Direct Blue 6	22 610	2602-46-2
Direct Red 28	22 120	573-58-0
Disperse Blue 1	64 500	2475-45-8
Disperse Orange 11	60 700	82-28-0
Disperse Yellow 3	11 855	2832-40-8
Dyestuffs classified as being allergenic		
Disperse Blue 3	61 505	2475-46-9
Disperse Blue 7	62 500	3179-90-6
Disperse Blue 26	63 305	
Disperse Blue 35		12222-75-2
Disperse Blue 102		12222-97-8
Disperse Blue 106		12223-01-7
Disperse Blue 124		61951-51-7
Disperse Orange 1	11 080	2581-69-3
Disperse Orange 3	11 005	730-40-5
Disperse Orange 37	11 132	
Disperse Orange 76	11 132	
Disperse Red 1	11 110	2872-52-8
Disperse Red 11	62 015	2872-48-2
Disperse Red 17	11 210	3179-89-3
Disperse Yellow 1	10 345	119-15-3
Disperse Yellow 9	10 375	6373-73-5
Disperse Yellow 39		
Disperse Yellow 49		

Additional dyestuffs classified as being allergenic by Oeko-Tex		
Disperse Blue 1	64 500	2475-45-8
Disperse Brown 1		23355-64-8
Disperse Yellow 3	11 855	2832-40-8

PPD has historically been used for dyeing textiles and has been used as a screening allergen for textile dye dermatitis (71). Nowadays, PPD is no longer used as a textile dye, but mainly as a hair dye. However, simultaneous patch test reactions to PPD and a range of textile dyes have frequently been reported (26,72). Besides the use of PPD in hair dye, several authors have reported contact allergy to PPD due to sensitization from temporary "tourist" tattoos (73-75), and in 2006 the illegal use of PPD in permanent black eyelash and eyebrow dye was described (76). In Sweden, PPD was forbidden in hair dye from 1943 until the Swedish entrance in EU 1995, but it is now permitted in hair dye products for use at concentrations ≤ 6% free base in the finished product (77). Chemically related substances, such as derivatives of PPD included in black rubber mix (BRM), have not been subjected to EU legislation and have not been used for dyeing textiles.

1.3 Disperse dyes

1.3.1 Properties and use

Disperse dyes have low solubility in water and were originally developed for the dyeing of cellulose acetate. The dyes are often mixed with a dispersing agent, e.g. lignin sulphonate (29), and then sold as a paste or as a powder. Their main use is in dyeing polyester but they can also be used to dye nylon, cellulose triacetate and acrylic fibres. They are not used to dye natural fibres (2,29). The dye is applied in a water dispersion, and the fine particle size ensures a large surface area, which aids uptake by the fibre. Most of the disperse dyes are azo dyes, but some are anthraquinones. The general structure of disperse dyes is a small, planar non-ionic molecule with polar functional groups such as $–NO_2$ and –CN. The shape makes it easy for the dye to "slide" between the polymer chains, and the polar groups improve the water solubility and dipolar bonding between dye and polymer, and affect the colour of the dye. However, they may be removed by rubbing and exposure to water (30,78).

Disperse dyes are mainly used for dyeing textiles, not only for clothes, but also for furnishing fabrics, car interiors and sports equipment. Furthermore, disperse dyes are used for dyeing fur, in leather processing, and for dyeing plastics. Disperse Red (DR) 1, DR 13, and DO 25 have all been found in toy products (79).

Occupational exposure to disperse dyes is seen in industry, particularly in the production of dyestuffs and the manufacture of synthetic textiles (4,80,81), but also in the handling of finished products in fabric shops, clothes shops, in laundries, and by dressmakers. Furthermore, working clothes and uniforms are often made of synthetic materials dyed with disperse dyes. Hairdressers belong to another group frequently exposed, not only to PPD but also to disperse dyes used in hair dyes. DB 7, DO 3, DR 11, Disperse (D) Brown 1 and D Violet 4 have been used in hair

dyes. Their use in hair dyes has been forbidden in Sweden since 2008 by the Swedish Medical Products Agency (77).

1.3.2 Contact allergy and allergic contact dermatitis

It is important to differentiate between contact allergy and ACD. Contact allergy, also known as "delayed contact hypersensitivity" or "type IV allergy", develops after the skin comes into contact with sensitizing substances, i.e. contact allergens (haptens). More than 3500 substances are known to cause contact allergy (82) and once an individual has become sensitized, the allergy remains throughout life. ACD is the clinical consequence when an individual with a contact allergy to a substance is exposed to the allergen in a concentration exceeding that individual's threshold. Thus, if a sensitized individual avoids contact with the allergen in question, or substances chemically related to the allergen (possible cross-reactants), he or she will not develop ACD.

A compound must meet at least two requirements in order to be classified as a hapten: it must be able to penetrate the skin, and it must be able to react with proteins in the skin to form antigens. To penetrate the skin, it must be a relatively lipophilic compound (log $P_{o/w}$>1) of low molecular weight, usually below 500-700 Dalton (82-84). The disperse dyes investigated in the present studies have values of log P from 2.5 to 5, and have molecular weights from 242 to 377 Dalton. To provoke an immune response, the hapten must bind to macromolecules in the skin once it has entered the epidermis. Many macromolecules, such as proteins, contain functional groups called nucleophiles, which are negatively charged. These groups can readily form covalent bonds with electrophiles, which are chemicals containing atoms that are positively charged (85). Consequently, most haptens possess electrophilic properties. Some molecules are electrophilic in themselves, while others act as prohaptens and require activation by metabolic processes and/or oxidation in contact with air and light to gain electrophilic properties (84). PPD is a prototype prohapten, which requires oxidation to a reactive metabolite to become allergenic (84). Among the amino acids present in proteins, lysine and cysteine are most often reported to be involved in skin sensitization, but other amino acids such as histidine, methionine and tyrosine also contain nucleophilic atoms that can react with electrophiles (86).

In contact allergy, a distinction is made between the sensitization phase, in which the immunological memory of the contact sensitizer is established, and the elicitation phase. In humans, the sensitization phase is considered to require 7 days to several weeks (84,87). In this phase, the hapten binds to proteins in the skin and forms antigens that are taken up by antigen-presenting cells called Langerhans cells. The Langerhans cells transport the antigen to the regional lymph nodes where it is presented to uncommitted T-cells that become activated (82). Activated T-cells

release cytokines, which leads to the proliferation and differentiation of the T-cells into hapten-specific memory cells, with effector or memory function, which are released into the blood circulation (84,87). Upon renewed contact with the hapten, i.e. the elicitation phase, Langerhans cells present the antigen to the allergen-specific memory and effector T-cells, which are circulating in the body. The allergen-specific T-cells become activated, proliferate and induce a cascade of inflammatory events in the area of skin exposed. Subsequently, an eczematous reaction usually develops 1-2 days after contact with the allergen (87,88). For some substances, however, the elicitation phase requires a much longer time, sometimes more than 2-3 weeks before ACD develops (84,89-91).

1.3.3 Contact allergy and allergic contact dermatitis from disperse dyes

As disperse dyes are mainly used for dyeing textiles, contact allergy and ACD due to disperse dyes arise from clothes and other textiles (18,21,28,92-95). Nowadays, underwear and sportswear are often made from synthetic textiles, dyed with disperse dyes. As they are close-fitting and are used during physical exercise leading to increased sweating and friction, there is a risk of sensitization and the elicitation of ACD resulting from the dyes used. Seat belt dermatitis from blue disperse dyes (96), contact allergy to disperse dyes in synthetic wigs (97) and to disperse dyes in plastic spectacle frames have been described in various case reports (98,99).

Disperse dyes should also be regarded as potential allergens in children with suspected contact sensitization (100-102). In a study carried out in the USA, diaper dermatitis in children with contact allergy to disperse dyes has been reported (103). Based on patch test results and the fact that the children improved with the use of dye-free diapers, the authors suspected that disperse dyes were used in the diapers, probably for aesthetic purposes.

Occupational contact allergy and ACD to disperse dyes in textile industry workers has been described in studies from Italy (4) and the USA (80). The authors in the American study stressed that workers who develop dermatitis, especially on their hands, should not only be patch-tested with the ordinary baseline series, but also with a textile series. As mentioned above, hairdressers also belong to a group exhibiting a high frequency of contact allergy and ACD (104). They often have hand eczema, and PPD is a common allergen in this group. Some disperse dyes with known sensitizing potential may still be used in hair dyes (e.g. D Violet 1). Simultaneous contact allergy to PPD derivatives in colour film developers and to several disperse dyes was reported in 1997 in a patient occupationally exposed to film developers (105). Furthermore, occupational ACD resulting from contact with disperse dyes in working clothes, including army uniforms and airline uniforms, has also been reported (106,107,108).

Anibarro et al. (109) described a patient working in a dressmaking factory, who developed widespread dermatitis due to airborne distribution while ironing clothes with a steam iron. Patch testing showed positive reactions to DB 106 and DB 124. The authors believed that the dyes could have been released from fibres by vaporization during ironing.

1.3.4 Sensitizing potential

At least 32 disperse dyes have been described as contact allergens (54). This constitutes roughly 2/3 of the textile dyes identified as allergens. Although disperse dyes are the most common sensitizers among textile dyes, there is little knowledge on their relative skin-sensitizing capacity, which could be used for risk assessment. Animal tests are required to establish whether a disperse dye is a contact allergen, and to measure its potential to cause skin sensitization. In the local lymph node assay (LLNA), the assessment of the proliferative response induced in the draining lymph nodes following exposure of mice to a test chemical is used to determine the sensitization potential (54). The concentration of the chemical required to cause a 3-fold increase in lymph node cell proliferation compared with concurrently vehicle-treated controls is known as the EC3 value.

In guinea pig tests, the sensitization potential of a chemical is determined by visual assessment of erythema and/or oedema following a challenge to the skin (54). As the whole process from sensitization to elicitation is involved, the guinea pig test methods mirror the clinical testing situation. Only guinea pig tests can be used to investigate cross-sensitivity between different chemicals.

In a study from 1989, in which 6 azo and anthraquinone dyes were tested with a guinea pig sensitization test, DB 1 and DB 124 were reported to be the strongest sensitizers, followed by DB 3, DO 3 and DR 1, while DY 3 was the weakest allergen (110). In a more recent study, DO 3 was also shown to be a significant contact sensitizer in guinea pigs (111). Although DY 3 was found to be a weak sensitizer, both in the study from 1989 (110) and in a more recent study using modified LLNA protocols in mice (112), it has been demonstrated to be a frequent allergen (93).

DB 106 has proven to be a strong allergen in guinea pig tests (113). When tested on mice, a mixed sample of DB 106 and DB 124 gave a moderate stimulation index (114), whereas Betts et al. reported that DB 106 (CI DB 106, 87% pure) had a relatively low EC3 value of 0.015% w/v, which is comparable to that found for 2,4-dinitrochlorobenzene (115,116). Corresponding values of EC 3 for PPD were found to be 0.07 and 0.15% w/v, when analysed at 2 different laboratories, also indicating PPD to be a strong sensitizer (117). These findings confirm the results of

clinical studies, indicating that both DB 106 and PPD are important allergens (95,118,119).

However, besides considering the sensitization potential and exposure, more information must be obtained on dye fastness in various types of fabric, dye migration and percutaneous penetration before risk assessments can be made for individual disperse dyes (115).

1.3.5 The prevalence of contact allergy

The true prevalence of contact allergy to disperse dyes in the general population is unknown, due to the fact that disperse dyes have not been included any epidemiological studies, or in studies where the prevalence of contact allergy in the general population has been estimated (120,121). Hatch and Maibach reported in their review article (93), that the highest prevalence in consecutively patch-tested dermatitis patients was 2.2% for DB 124 (122) and 1.7% for DO 3 (123). In contrast, the prevalence among patients known to be sensitized to textile dyes was found to be 36% for DB 124 and 28% for DO 3 (124). Some dermatologists claim that contact allergy to disperse dyes is decreasing (13), whereas data from more recent studies (Table 4) show that the frequency of disperse dye allergy has increased (10,125), underlining the need for further investigations in this field. The results of a retrospective study carried out in Israel showed that improving the awareness of clinical dermatologists led to a reduction in the average duration of textile dermatitis until diagnosis from 17.3 months to 10.6 months (10). The prevalence of disperse dye contact allergy varies depending on the population tested. The prevalence reported in studies in various populations can be found in Table 4.

Table 4. *Prevalence of disperse dye contact allergy in consecutively patch-tested dermatitis patients (screening patch testing), patients suspected of having textile dye allergic contact dermatitis (aimed patch testing), and patients known, or thought likely, to be sensitized to textile dyes (known contact allergy).*

Disperse dye	Conc. (%)	Population	No. patients tested	No. patients allergic	%	Year of publication, country	Ref.
D Blue 106	1	Screening	5514	196	3.6	2002, Italy	(125)
	1.0	Aimed	159	16	9.8	1992, Belgium	(126)
	1	Aimed	577	34	5.9	2003, NL	(127)
D Blue 124	1	Screening	576	11	1.9	1990, Italy	(128)
	1	Screening	6478	193	3.0	2002, Italy	(125)
	1	Aimed	577	29	5.0	2003, NL	(127)
	1	Known	100	36	36	1991, Italy	(124)
D Blue 35	1.0	Aimed	159	6	3.8	1992, Belgium	(126)
	1	Aimed	577	7	1.2	2003, NL	(127)
	1	Known	98	5	5.1	1991, Italy	(124)
D Yellow 3	1	Screening	576	3	0.5	1990, Italy	(128)
	1	Screening	6478	95	1.5	2002, Italy	(125)
	1	Aimed	577	8	1.4	2003, NL	(127)
	1	Known	100	24	24	1991, Italy	(124)
D Orange1	1.0	Aimed	159	2	1.3	1992, Belgium	(126)
	1	Aimed	577	19	3.3	2003, NL	(127)
	1.0	Aimed	644	3	0.5	2004, Israel	(10)
D Orange 3	1	Screening	576	5	0.9	1990, Italy	(128)
	1	Screening	6478	143	2.2	2002, Italy	(125)
	1	Aimed	577	29	5.0	2003, NL	(127)
	1	Known	100	28	28	1991, Italy	(124)
D Red 1	1	Screening	576	6	1.0	1990, Italy	(128)
	1	Screening	6478	67	1.4	2002, Italy	(125)
	1.0	Aimed	159	2	1.3	1992, Belgium	(126)
	1	Aimed	577	7	1.2	2003, NL	(127)
	1	Known	100	29	29	1991, Italy	(124)
D Red 17	1.0	Aimed	159	6	3.8	1992, Belgium	(126)
	1	Aimed	577	3	0.5	2003, NL	(127)
	1	Known	98	20	20.4	1991, Italy	(124)
D Black 1	1	Screening	569	1	0.2	1991, Italy	(23)
	1	Known	98	12	12.2	1991, Italy	(124)
D Yellow 9	1.0	Aimed	159	2	1.3	1992, Belgium	(126)
	1	Aimed	577	3	0.5	2003, NL	(127)
	1	Known	98	11	11.2	1991, Italy	(124)
D Orange 13	1.0	Aimed	159	2	1.3	1992, Belgium	(126)
	1	Aimed	577	3	0.5	2003, NL	(127)
D Blue 85	1.0	Aimed	159	2	1.3	1992, Belgium	(126)
	1	Aimed	577	2	0.3	2003, NL	(127)
D Blue 153	1.0	Aimed	159	3	1.9	1992, Belgium	(126)
	1	Aimed	577	3	0.5	2003, NL	(127)
D Brown 1	1.0	Aimed	159	4	2.5	1992, Belgium	(126)
	1	Aimed	577	8	1.4	2003, NL	(127)

Conc., concentration; NL, the Netherlands.

1.3.6 Skin manifestations

ACD resulting from textile dyes often has the clinical features typical of eczema (2,125) and may progress to more generalized lesions if further contact with the allergen is not avoided (129). Two different clinical types of disperse dye dermatitis can be identified: an eczematous and a more uncommon oedematous plaque type, mainly associated with DB sensitization (18). However, skin manifestations resulting from contact allergy to dyed clothing and other textiles may go unrecognised if the clinical picture resembles that of other disorders such as seborrhoeic dermatitis, atopic dermatitis, mycosis, or neurodermatitis (124) rather than textile dermatitis (18,108). Less common manifestations such as urticaria, erythema multiforme, and toxicoderma-like eruptions due to textile-related contact dermatitis have also been described (18,130). Pigmented purpuric contact dermatitis has been reported, especially in patients with contact allergy to DB 106 and DB 124 (131,132). The distribution of the dermatitis may be typical, with eruptions starting in the axillae, apart from hair-covered areas, but with patches on the anterior and posterior chest, in typical cases sharply limited by the underwear. The neck and the bend of the arms may also be affected, and also the inner posterior thighs, popliteal fossae and lower legs. Dermatitis may be limited to areas where friction is high between skin and garment and/or where a significant amount of moisture is present (2). Allergy to dyes in socks, stockings and tights often starts on the dorsa of the feet. Hand dermatitis usually affects those occupationally exposed, although non-occupational ACD on the hands has also been reported (124). However, atypical distributions of dermatitis, for example, on the face (13,125), can also be a sign of textile dermatitis.

1.3.7 Patch testing

Disperse dyes are not included in most baseline patch test series, but several disperse dyes known to cause contact allergy are included in commercially available textile patch test series. As many disperse dyes are potential contact allergens, the possibility of using some compounds in the testing of dermatitis patients to demonstrate a textile dye allergy would be helpful (30). Mixes of several disperse dyes have been used for patch testing in various studies in order to identify patients with contact allergy to textile dyes (133-137). The mixes used in some studies are listed in Table 4. However, the frequency of allergic patients in the various studies can not be compared because of different inclusion criteria. One common conclusion of all these studies was that further investigations were needed, with regard to both which disperse dyes should be included in testing, and the concentrations of the individual ingredients, before deciding whether a particular textile dye mix (TDM) should be added to the baseline series.

Table 5. *Disperse dye mixes used in different studies.*

Mix no.	Components	Conc. (%)	No. tested	No. allergic	%	Year of publication, country	Ref.
1	DY 3/DO 3/ DR 1/DB 124	0.25/0.25/ 0.25/0.1	31*	31		1994, Italy	(133)
2	DY 3/DO 3/ DR 1/DB 124	0.25/0.25/ 0.25/0.1	67*	60		1995, Italy	(135)
3	DB 35/DB 106/ DO 3	1/1/1	78	2	2.6	1992, Portugal	(134)
4	DR 1/DR 17/ DY 3/ DB 35/ DB 124	#	78	2	2.6	1994, Portugal	(134)
5	DR 1/DR 17/ DY 3/ DB 35/ DB 124/DO 3/ DB 3/DO 37	#	78	2	2.6	1994, Portugal	(134)
6	DB 124/DR 1/ DO 3/DY 3	1/0.25/ 0.25/0.5	31*	26		1998, Italy	(122)
7	DB 106/DB 124	1/1	1108¤	52	4.7	2001, Germany	(138)
			3041	40	1.3	2003, Germany	(136)
			6856	67	1.0	2006, UK	(137)
			24980	337	1.4	2007, Germany	(118)

* Patients with known contact allergy to at least one of the dyes. ¤ Aimed testing. # Concentrations not given in the article.

DB, Disperse Blue; DO, Disperse Orange; DR, Disperse Red; DY, Disperse Yellow.

2. AIMS

The general aim of the work presented in this thesis was to investigate the clinical and chemical aspects of patch testing with a textile dye mix consisting of 8 disperse dyes, Disperse Blue 35, Disperse Blue 106, and Disperse Blue 124, Disperse Yellow 3, Disperse Orange 1 and Disperse Orange 3, Disperse Red 1 and Disperse Red 17. More specifically, the purposes of the studies were to investigate:

- the prevalence of contact allergy to a textile dye mix in a baseline patch test series,

- whether a textile dye mix could be used as a potential marker of contact allergy to the 8 disperse dyes,

- the relationship between self-reported skin manifestations due to textiles and contact allergy to the textile dye mix, p-phenylenediamine, and rubber chemicals related to p-phenylenediamine,

- the chemical purity and concentration of purified dyes in the 8 disperse dyes used in the mix,

- the content and purity of patch test preparations stated to contain the 8 disperse dyes, used at dermatology departments in different countries,

- the elicitation potential of commercial and purified Disperse Blue 106 and Disperse Blue 124, and

- the significance of impurities in patch test preparations of Disperse Blue 106 and Disperse Blue 124 with regard to contact allergy.

3. MATERIALS AND METHODS

3.1 Subjects

A total of 5105 consecutively patch-tested patients, 3031 females and 2074 males, were enrolled in the four studies focusing on the clinical studies of contact allergy to disperse dyes (Papers I-III & V). The first study (Paper I) was performed at the Department of Occupational and Environmental Dermatology, Malmö University Hospital, Malmö, Sweden, while the remaining two studies (II-III) were performed at this department and at the Department of Dermatology, Katholieke Universiteit Leuven, Belgium. The patch test results from the 982 patients who participated in Study II were also included in Study III. In Study II, 497 women and 361 men answered a questionnaire.

The 21 patients who participated in Study V were recruited from among the patients with contact allergy to DB 106 and/or DB 124 identified in the first three studies. Demographic data on all patch-tested patients are given in Table 6, and Studies II and III are outlined in Figure 4.

Table 6. *Demographic data on all patients patch tested in the four clinical studies.*

Study	**Number of patients**	**Women** (%)	**Men** (%)	**Mean age** (years)	**Age range** (years)
I	3325	58.4	41.6	46.5	10-90
II	982	57.8	42.2	42.8	13-94
II	858 *	57.9	42.1	43.4	13-94
III	1780 **	61.2	38.8	43.2	13-94
V	21	85.7	14.3	47.8	26-75

* The number of patients in Study II who answered the questionnaire and were patch tested.

** Including the 982 patients in Study II.

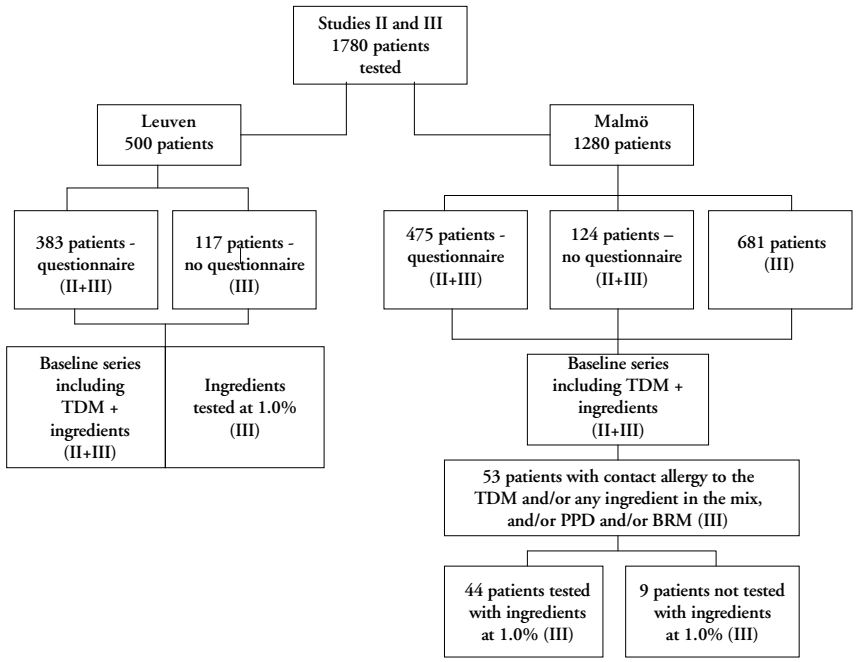


Figure 4. *The logistics of Studies II and III, including the number of patients who participated at the 2 departments.*

3.2 Chemicals and patch test preparations

The main chemicals and patch test preparations used in Studies I-V are listed in Tables 7 and 8. The concentration of each substance diluted in acetone is given in % w/v, and the concentration of a substance mixed in petrolatum (pet.) is given in % w/w. The molecular structures and main properties of the 8 disperse dyes used in the mix are given in Figure 5, and those of PPD and the 3 components in BRM in Figure 6.

Table 7. *Main chemicals with manufacturers/suppliers.*

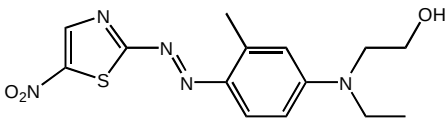
Chemical	Manufacturer/supplier	Paper
Petrolatum	Apoteksbolaget, Sweden	I-IV
Acetonitrile	Scharlau Chemie S.A., Spain	IV
Acetonitrile for TLC systems	Lab-Scan, Ireland	IV, V
Acetone	Scharlau Chemie S.A., Spain	IV, V
Chloroform	Scharlau Chemie S.A., Spain	IV, V
Ethanol	Kemetyl, Sweden	IV
Heptane	Mallinckrodt Baker B.V., the Netherlands	IV
Triethylamine	Fisher Scientific, UK	IV
Disperse Blue 35	Chemotechnique Diagnostics, Sweden	I-V
Disperse Blue 106	Chemotechnique Diagnostics, Sweden	I-V
Disperse Blue 124	Chemotechnique Diagnostics, Sweden	I-V
Disperse Yellow 3	Chemotechnique Diagnostics, Sweden	I-V
Disperse Orange 1	Chemotechnique Diagnostics, Sweden	I-V
Disperse Orange 3	Chemotechnique Diagnostics, Sweden	I-V
Disperse Red 1	Chemotechnique Diagnostics, Sweden	I-V
Disperse Red 17	Chemotechnique Diagnostics, Sweden	I-V

TLC, thin-layer chromatography.

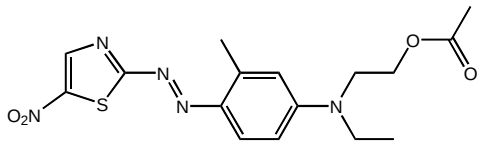
Table 1. *Main patch test preparations with producers/suppliers.*

Patch test preparation	Producer/supplier	Vehicle	Concentration (% w/w pet. %w/v in ac.)	Paper
Textile dye mix	Dept. of Occupational and Environmental Dermatology, Malmö, Sweden	pet.	3.2%	I-III
The 8 separate disperse dyes used in the mix	Dept. of Occupational and Environmental Dermatology, Malmö, Sweden	pet.	0.1% or 0.5%	I-III
			1.0%	IV
		ac.	1.0 - 10^{-6}%	V
The 8 disperse dyes	Chemotechnique Diagnostics, Sweden	pet.	1.0%	II, III
p-Phenylenediamine, Malmö	Chemotechnique Diagnostics, Sweden	pet.	1%	I-III
p-Phenylenediamine, Leuven	TROLAB, Germany	pet.	1%	II, III
Black rubber mix	Chemotechnique Diagnostics, Sweden	pet.	0.6%	I-III

ac., acetone; pet., petrolatum.

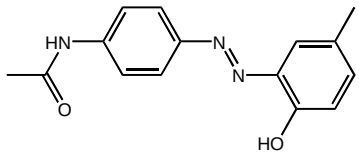


Disperse Blue 106
CAS: 68516-81-4; CI: 111935; MW: 335;
log $P_{o/w}$: 2.92

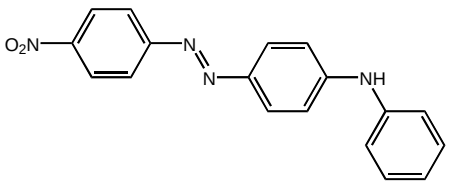


Disperse Blue 124
CAS: 15141-18-1; CI: 111938; MW: 377;
log $P_{o/w}$: 3.74

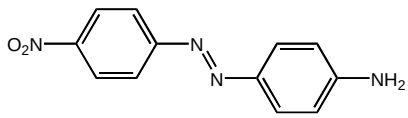
Disperse Blue 35
Chemical formula not specified
according to Chemical Abstracts.
CAS: 12222-75-2; CI: not found



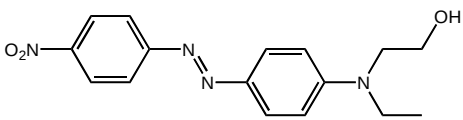
Disperse Yellow 3
CAS: 2832-40-8; CI: 11855; MW: 269;
log $P_{o/w}$: 3.48



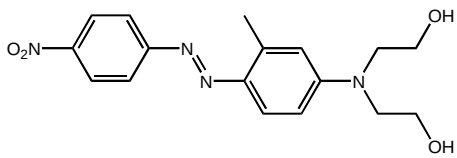
Disperse Orange 1
CAS: 2581-69-3; CI: 11080; MW: 318;
log $P_{o/w}$: 5



Disperse Orange 3
CAS: 730-40-5: CI: 11005; MW: 242;
log $P_{o/w}$: 2.59

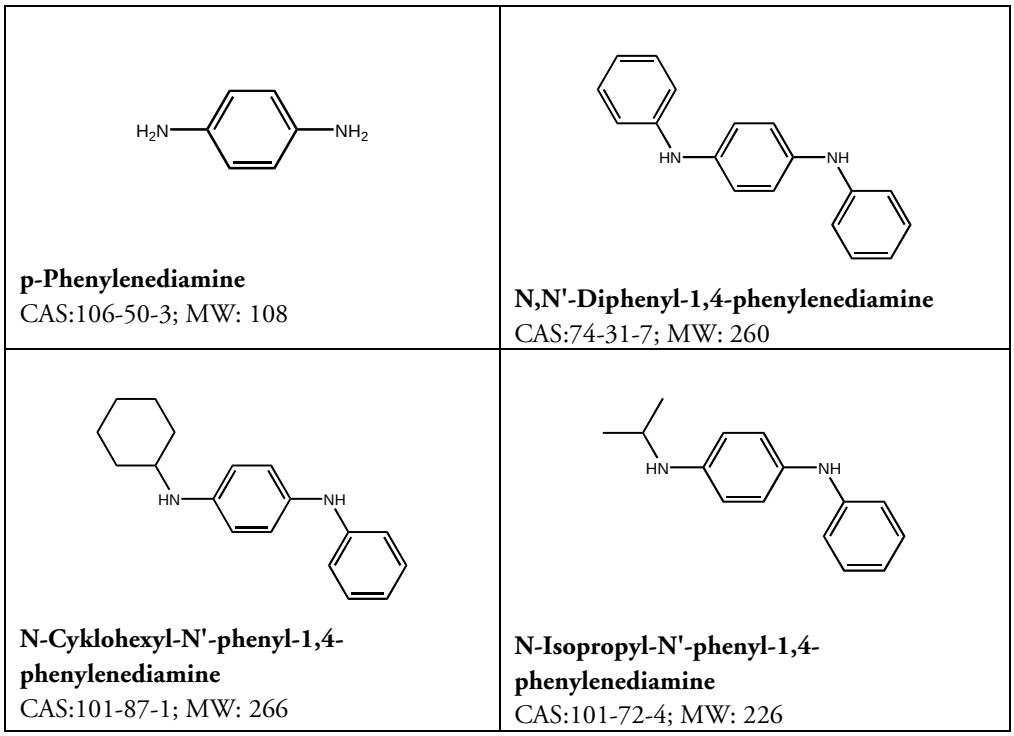


Disperse Red 1
CAS: 2872-52-8; CI: 11110; MW: 314;
log $P_{o/w}$: 3.25



Disperse Red 17
CAS: 3179-89-3; CI: 11210; MW: 344;
log $P_{o/w}$: 2.5

Figure 5. *The chemical structures, Chemical Abstract Service (CAS) and Colour Index (CI) numbers, together with the molecular weights (MW) and the log $P_{o/w}$ values of the disperse dyes used.*



p-Phenylenediamine
CAS:106-50-3; MW: 108

N,N'-Diphenyl-1,4-phenylenediamine
CAS:74-31-7; MW: 260

N-Cyklohexyl-N'-phenyl-1,4-phenylenediamine
CAS:101-87-1; MW: 266

N-Isopropyl-N'-phenyl-1,4-phenylenediamine
CAS:101-72-4; MW: 226

Figure 6. *The chemical structures, Chemical Abstract Service (CAS) numbers, and the molecular weights (MW) of p-phenylenediamine and the 3 components in black rubber mix.*

3.3 Patch testing

The consecutively patch-tested dermatitis patients in Studies I and II/III were all tested with the baseline series used at the respective departments, including a mix of 8 disperse dyes consisting of DB 35, DY 3, DO 1, DO 3, DR 1, and DR 17 (at 0.5% w/w pet.), and DB 106 and DB 124 (at 0.1% w/w pet.), giving a total concentration of 3.2%. In Study I, the patients with contact allergy to the TDM at the first patch test reading were also tested with its ingredients at the same concentrations as in the mix. In Studies II/III the Leuven patients were tested simultaneously with the TDM, with its ingredients at the same concentrations as in the mix, and at 1.0% w/w (pet.) in the baseline series. The Malmö patients who were found to be allergic to at least 1 of the test preparations (the TDM, any of the 8 ingredients in the TDM, PPD or BRM) at the first patch test reading were additionally tested with the 8 disperse dyes at the higher concentration of 1.0% (Figure 4). In Study V the 21 patients were patch tested with dilution series of commercial and purified DB 106 and DB 124 prepared at the department in Malmö. About 20 mg of each disperse dye was accurately

weighed and dissolved in acetone, yielding a 1.0% w/v preparation. From this stock solution further dilutions, from 10^{-1} to 10^{-6} (0.10-0.0000010)% w/v, were prepared.

3.3.1 Patch testing with thin-layer chromatograms

TLC is a method used to separate the components in a chemical or a mixture of chemicals. The method is based on a stationary phase, e.g. a silica gel on a glass plate, an aluminium sheet or a plastic film, and an eluent as the mobile phase, e.g. acetone or acetonitrile. If the sample consists of more than 1 chemically defined substance, the eluent will transport the different components different distances along the plate, giving rise to bands of separate spots. In Study V the TLC technique was used for patch testing of individual patients with known contact allergy to DB 106 and DB 124 (139). To separate the ingredients in acetone solutions of DB 126 and DB 124, thin-layer chromatograms were prepared on plastic films (TLC plastic roll 500×20 cm silica gel 60F254 from Merck KgaA, Darmstadt, Germany). The preparation of the chromatograms is described in detail in Paper V. The first patients were tested with thin-layer chromatograms obtained with an eluent containing a mixture of chloroform and acetonitrile 86/14 (v/v) as the mobile phase (chromatograms A), but the proportions were then modified to obtain better separation of the spots. When the mixture was changed to 70/30 (v/v) chloroform and acetonitrile, visual inspection showed that less substance remained on the application spot and bands of well-defined and separated spots were obtained (chromatograms B).

3.3.2 Patch test technique

Patch testing was performed on each patient's back, according to the routine at the respective departments. The 4605 patients in Malmö (Studies I-III & V) and the 10 Leuven patients in Study V were tested with Finn Chambers® (diameter 8 mm, Epitest Ltd, Tuusula, Finland) on Scanpor® tape (Norgesplaster A/S, Vennesla, Norway), while the 500 patients in Leuven (Studies II/III) were patch tested with Van der Bend Square Chambers® (Brielle, the Netherlands) applied to the back with Micropore(tm) tape (3M Health Care, Borken, Germany) and fixed with Mefix® (Mölnlycke Health Care, Göteborg, Sweden). In Studies I and II/III petrolatum preparations were applied in each test chamber and in Study V 15 µl of the test solution was applied with a micropipette to the filter paper disc in each test chamber. The chambers were left on the patient's back for 2 days and readings were performed in Malmö on day 3 or 4 and on day 7 or 8 (Studies I-III), and in Leuven on day 2, day 4, and sometimes on day 7 (Studies II/III). In Study V the patients were also patch tested with the thin-layer chromatograms. The chromatograms were applied to the patient's back with Scanpor® tape and left for 2 days; readings were performed on day 3 and on day 6 or 7. The observations from both readings were recorded in the

first study (Study I) and Study V, while the observation on day 3 or 4 was recorded in Studies II and III.

3.3.3 Evaluation of patch tests

The patch test reactions were scored according to the guidelines of the International Contact Dermatitis Research Group (140): – = negative; (+)/? = faint erythema; + = erythema, infiltration, possibly papules; ++ = erythema, infiltration, papules, possibly vesicles; +++ = intense erythema, infiltration and vesicles. The minimum criterion for a positive patch test is homogeneous erythema and infiltration, i.e. +. Regarding the evaluation of patch test reactions in the last study (Study V), additional grading was used when scoring the positive reactions, as strong + and ++ reactions were graded +(+) and ++(+), respectively (141).

3.4 Questionnaire

In Study II the patients were interviewed, before patch testing, by the test personnel using a questionnaire, to obtain information on past and present skin problems related to textiles and exposure to textile dyes and chemically related substances such as PPD and BRM. The questions are listed in Table 9.

Table 9. *The questions in the questionnaire used in Study II.*

Have you ever had a rash/itch that you suspect is caused by textiles?	
If yes, which type of textile material?	
Wool	Silk
Cotton	
Synthetic material	
If yes, where were your skin problems from textiles located?	
Face	Arms
Scalp	Hands
Neck	Around groin
Trunk	Leg
Bend of arms	Hollow of the knee
Around armpit	Feet
Have you had eczema as a child?	
Have you coloured your hair?	
Have you had a tourist tattoo made on you?	
Do you work or have you worked with textiles	
a) in a dye works, textile factory, manufacture of textile dyes, supplier of textile dyes?	
b) where the finished textile is handled, e.g. dressmaker, in a fabric shop, clothes shop, laundry?	

3.5 Chemical investigations

In Study IV, the concentration of the 8 disperse dyes in patch test preparations used in testing at our department in Malmö and at 12 other dermatology departments in 10 countries around the world was determined with high performance liquid chromatography (HPLC). These concentrations were compared with reference substances. The petrolatum preparations had to be extracted before HPLC analysis.

3.5.1 Reference substances

As there were no disperse dye reference substances available for purchase they had to be isolated from the corresponding commercial dyes at the laboratory in our department in Malmö. For each of the disperse dyes, reference substances were isolated with HPLC and identified with mass spectrometry (MS) at the Malmö department. The purity of the reference substances was investigated with nuclear magnetic resonance spectrometry at Lund University. Several fractions were identified in DB 35, and it was not possible to isolate or identify a specific reference substance for this disperse dye. The purity of the other reference substances was >99%, except for DO 3, which had a purity of >97%. These purified substances were used as reference substances to which the commercial patch test preparations of the disperse dyes were compared in Study IV, and also for preparing the dilution series of purified DB 106 and DB 124 used in Study V.

3.5.2 Preparations of samples from patch test preparations

The extraction procedure for the patch test preparations is described in detail in Paper IV. Triplicate samples were taken from each preparation investigated to evaluate the homogeneity of the patch test preparation. About 0.1-0.5 g of the preparation was accurately weighed and placed in a test-tube to which heptane was added to dissolve the petrolatum preparation. The dye was gradually extracted and separated from the heptane phase by adding a solution of ethanol/acetone/water. The ethanol/acetone/water solution containing the dye was then evaporated to dryness. The residue was dissolved in 1.0 ml acetone and the solution was analysed using HPLC.

To evaluate the reproducibility of the work-up procedure, the recovery of the extraction was investigated. At least 3 preparations and subsequent extractions were made from each dye. The procedure is described in Paper IV, and the mean recovery of the 7 analysed disperse dyes is given in Table 10.

Table 10. *The mean recovery of the 3 preparations of each disperse dye.*

	DB 106	DB 124	DY 3	DO 1	DO 3	DR 1	DR 17
Mean recovery (% of stated value)	92.4	88.5	99.5	95.4	96.1	92.0	94.7
C.V. (%)	18.1	17.4	7.1	5.1	9.3	21.7	9.3

C.V., coefficient of variation.

3.5.3 High performance liquid chromatography

As with TLC, HPLC is also a method of separating chemical components based on the fact that the individual components are distributed differently when carried through a stationary phase by a mobile phase. Separation of the disperse dyes using HPLC involved a non-polar stationary phase and a polar mobile phase, containing a solvent of acetonitrile and water. The HPLC system and the linear gradient elution of the solvents used for the HPLC analysis are described in Paper IV. The concentration of the disperse dyes in the investigated patch test preparations was determined by comparing the peak area produced by a known concentration of the disperse dye reference substance with the peak area of the corresponding peak from the sample. The identity of the substance producing a certain peak was determined by the retention time and UV spectrum.

Repeatability of the HPLC method

In order to investigate the repeatability of the HPLC method a sample was taken from a patch test preparation representing each of the disperse dyes except DB 35. Triplicate samples of the acetone solutions were diluted and analysed using analytical HPLC. The analyses showed a coefficient of variation varying from 0 to 0.39%.

3.5.4 Thin-layer chromatography

In Study IV TLC was used to investigate the acetone solutions obtained from the disperse dye patch test preparations collected from various dermatology departments. TLC silica gel plates (TLC plates 20×20 cm with silica gel 60 F 254 on glass from VWR International, Stockholm, Sweden) were used to prepare the chromatograms. Fifty µl of the acetone solution from the work-up procedure was deposited on the lower part of the silica gel plate. The plate was placed in a glass beaker containing an eluent consisting of a mixture of chloroform and acetonitrile 86/14 (v/v). The acetone solutions of DB 106 and DB 124 were also analysed with an eluent containing a mixture of chloroform, acetonitrile and triethylamine 75/20/5 (v/v/v), to obtain better separation of the spots. The thin-layer chromatograms were inspected in visible light and in UV light (254 and 366 nm), and each patch test preparation was

compared with the TLC pattern of the corresponding disperse dye reference substance. The procedure is described in detail in Paper IV.

3.6 Recording of reactions

In Study I the sites of dermatitis were documented before the patients were tested. To compare the results of the patch-test-positive population to all tested patients we used Daluk, a data-based registration system in which age, gender, contact allergies and site of dermatitis are recorded (142). In Study II the standardized questionnaire was answered by the patients before patch testing to investigate whether patients with contact allergy to the TDM and related substances reported clinical signs and symptoms from textiles more often. Microsoft Office Excel 2003 was used to document the patients' answers to the questionnaire.

3.7 Ethics

The study described in Paper V was approved by the Regional Ethics Review Board in Lund, Sweden, and conducted in accordance with the ethical standards specified in the Declaration of Helsinki. All patients gave informed written consent to participate in the study.

3.8 Statistical calculations

In Studies II and III the results were analysed using SPSS version 12.0 (SPSS Inc. Chicago, IL, USA). The following statistical methods were used: Fisher's exact two-sided test (Studies I, II, III & V), the McNemar test (Study III), and in Study II odds ratios (OR), 95% confidence intervals (C.I.) and P values for a positive answer to the question concerning textile-related skin problems, calculated in a multiple logistic regression analysis. Two-sided P values < 0.05 were considered to be statistically significant.

4. RESULTS

The results of the 5 studies are described in detail in the corresponding papers. The results in the different studies will be compared and commented on briefly in this section.

4.1 Patch testing

As the results from the patients participating in Study II were combined with the results from Study III, the patch test results in Studies I and III are compared (Figure 7). Of 5105 consecutively patch-tested dermatitis patients in Studies I and III, 85 (1.7%) reacted to TDM 3.2% w/w (pet.). In Study I, 1.5% of the 3325 patients showed positive reactions to the dye mix, while 2.0% of the 1780 patients in Study III reacted to the mix (Study I vs. Study III, $P = 0.13$). Contact allergy to PPD was found in 2.0% of the patients in Study I and 3.9% of the patients in Study III ($P < 0.001$), while 0.6% of the patients in Study I reacted to BRM compared to 1.0% in Study III ($P = 0.11$). The percentage of patients in Study III with contact allergy to the TDM, PPD and BRM in Malmö and in Leuven are shown in Figure 7, where it can be seen that contact allergy to PPD was significantly more common in Leuven than in Malmö ($P < 0.001$). No such differences were seen regarding contact allergy to TDM or BRM (both $P > 0.3$). The number of patients among the 5105 tested showing a positive reaction to patch tests for the TDM, PPD and BRM, individually and combined, is shown in Figure 8.

In the TDM-positive patients, the most frequent contact allergy to the ingredients in the mix, tested at the same concentrations as in the mix, in both Studies I and III was DO 1, followed by DY 3. Simultaneous contact allergy to DB 106 and DB 124 in the TDM-positive patients was found in 2/9 (Study I) and 6/6 (Study III) patients with contact allergy to any of these dyes. In the first study, 2.1% of the TDM-positive patients reacted to DO 3, tested at the same concentration as in the mix, compared with 17.1% in Study III ($P = 0.020$). However, the chemical investigations described in Paper IV revealed that the DO 3 tested at the concentration used in the mix, in both Studies I and III, in reality was DO 31. Hence, the patch test preparation labelled DO 3 0.5% will henceforth be described as “DO 3”.

In Study I, 36% of the TDM-positive patients tested negatively to the separate ingredients when tested at the same concentrations as those used in the mix; which can be compared with 29% among the TDM-positive patients in Study III ($P = 0.15$).

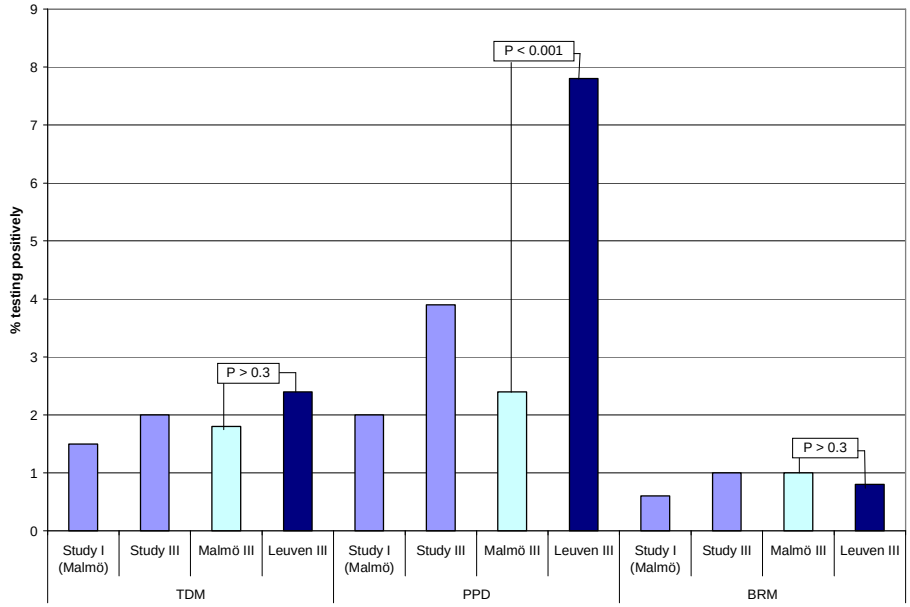


Figure 7. *Contact allergy to the TDM, PPD and BRM in 5105 patch-tested patients in Studies I and III. The results obtained from testing in Malmö and Leuven are also shown separately. P values for comparisons between the patch test result in Malmö and Leuven in Study III are also given.*

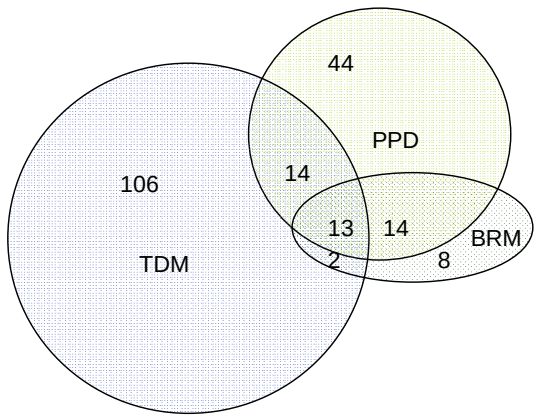


Figure 8. *The pattern of concomitant contact allergy to the TDM, PPD and BRM in the 5105 patch-tested patients.*

No comparison can be made between the numbers of TDM-negative patients with contact allergy to any ingredient in the two studies, as the TDM-negative patients in Study I were not patch tested with the separate ingredients in the mix. The percentage of patients in both studies (I+III) with contact allergy to the TDM, TDM and PPD, and TDM and BRM, and with simultaneous reactions to the ingredients in the TDM, can be seen in Figure 9.

Contact allergy to the ingredients in the TDM, tested at the concentrations used in the mix, in the patients from Leuven and Malmö (Paper III) can be seen in Figure 10. Although not statistically significant, a tendency was seen towards a higher frequency of patients with contact allergy to "DO 3" and DR 17 in Leuven than in Malmö.

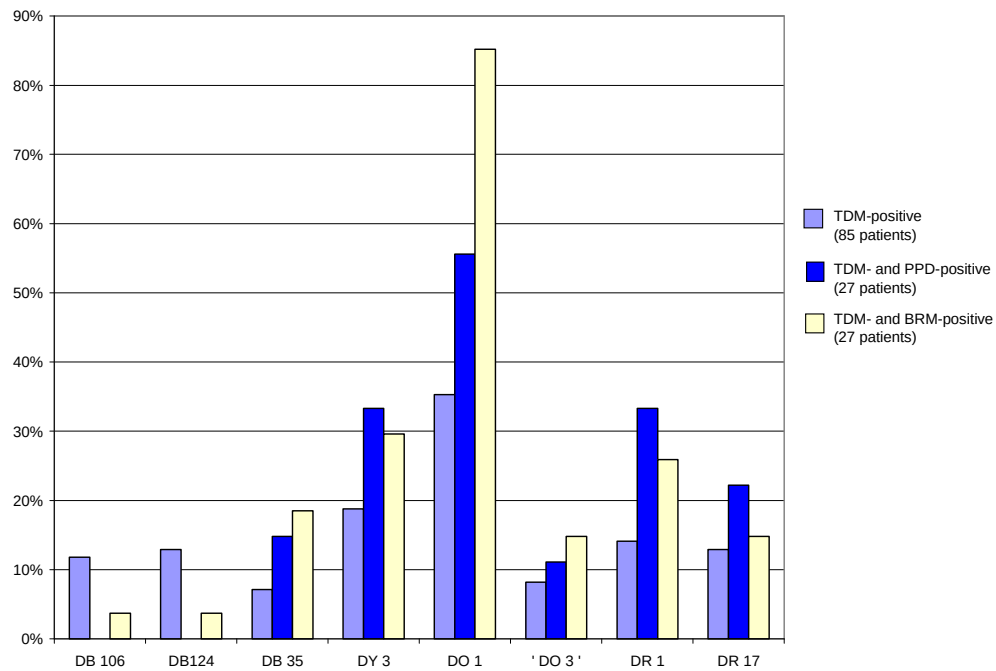


Figure 9. *The total proportion (%) of patients in Studies I and III with contact allergy to the TDM, TDM and PPD, TDM and BRM, and with simultaneous contact allergy to the separate ingredients in the TDM.*

4.1.1 Patch testing with the disperse dyes at different concentrations

The main aim of Study III was to investigate whether patch testing with the TDM identified patients with contact allergy to its ingredients, when they were

simultaneously patch tested with the TDM and with the ingredients tested in the same vehicle and at the same concentration as used in the mix. The results are described in detail in Paper III. The 500 patients in Leuven were simultaneously tested with the TDM, the separate ingredients at the same concentrations as in the mix, and at a higher concentration of 1.0% w/w (pet.). The separate results from the patch testing of the Leuven patients can be seen in Figure 11.

Among the 500 patients, simultaneous testing at a 10 times higher concentration revealed 5 and 4.5 times more patients with contact allergy to DB 106 and DB 124, respectively. Testing DO 3 at the higher concentration revealed 18 patients with contact allergy to DO 3, compared with 4 patients with contact allergy to "DO 3" when tested at the same concentration as in the mix.

4.1.2 Degree of hypersensitivity

In Study I the reactivity to the 8 ingredients of the dye mix varied. Most of the test reactions to DB 106, DB 124 and DR 17, tested at the concentration used in the mix, were weak, whereas most of the reactions to DO 1 were strong. The pattern of reactivity to the 8 components in the TDM can be seen in Paper I, Figure 5. In Study V, the patients were patch tested with dilution series of commercial and purified DB 106 and DB 124. A total of 16/21 and 15/20 patients were allergic to the dilution series of the commercial DB 106 and DB 124, respectively, and 10 patients reacted to each of the dilution series of the corresponding purified disperse dyes. Two patients were allergic to the lowest concentrations, i.e. 0.0000010% (0.010 µg/ml), of the purified DB 106. Only 1 of these patients was tested with the dilution series of DB 124, but this patient also reacted down to 0.0000010% of the purified DB 124. No corresponding patch testing with dilution series of purified and/or commercial DO 1 or of the remaining disperse dyes in the mix was performed in the present studies.

4.2 Testing with thin-layer chromatograms

The patients patch tested with the thin-layer chromatograms in Study V showed a varying pattern, described in detail in Paper V. The patients patch tested with chromatograms A all reacted to both the main spots corresponding to the purified DB 106 and DB 124 and to spots closer to the application area (Figure 12), whereas 10/18 and 10/16 patients patch tested with chromatograms B reacted to the DB 106 and DB 124 chromatograms, respectively. Among these patients, 4/10 (DB 106) and 5/10 (DB 124) did not react to the main spot but to other spots, including a pink spot located close to the application area on chromatograms B (Figure 13). Furthermore, 3 of the patients patch tested with chromatograms B showed a reaction to at least 1 of the chromatograms only at the second reading on day 7. Patch test

reactions to DB 106 and DB 124 (chromatograms A and B) and a close-up view of an allergic reaction to the pink spot can be seen in Figures 12-14.

The acetone solutions of DB 106 and DB 124 used for the thin-layer chromatogram testing in Study V were prepared according to the procedure described in Section 3.5.2. Ten patients in Study V were additionally patch tested with a sample of the heptane fraction from the extraction of these dyes, in order to investigate whether substances with allergenic properties remained in this fraction. No allergic reactions were observed among these patients. One of the patients was excluded due to widespread allergic reactions, which made it impossible to discern the reactions to separate allergens.

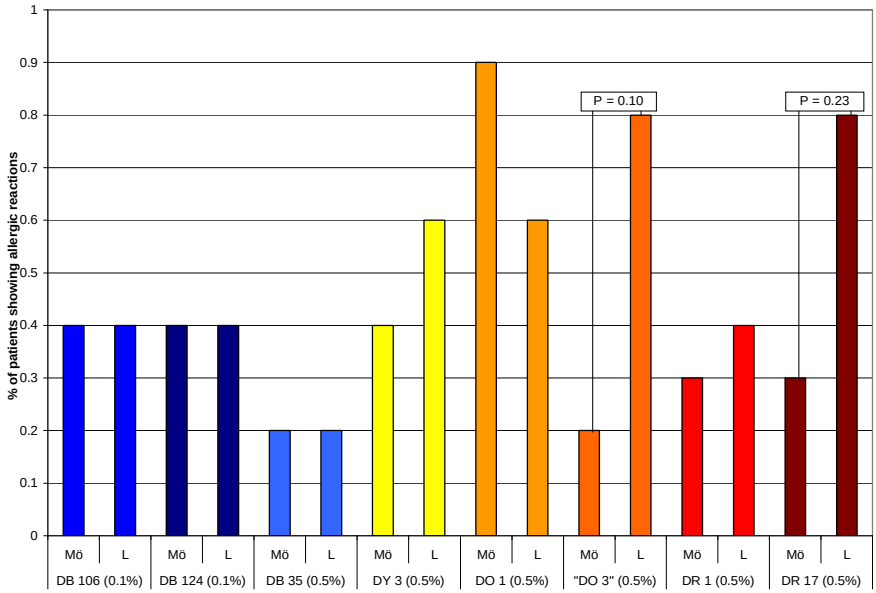


Figure 10. *The percentage (%) of patients in Malmö (Mö) and Leuven (L) with contact allergy to the separate ingredients tested at the concentration in the mix. P values < 0.3 are indicated in the figure.*

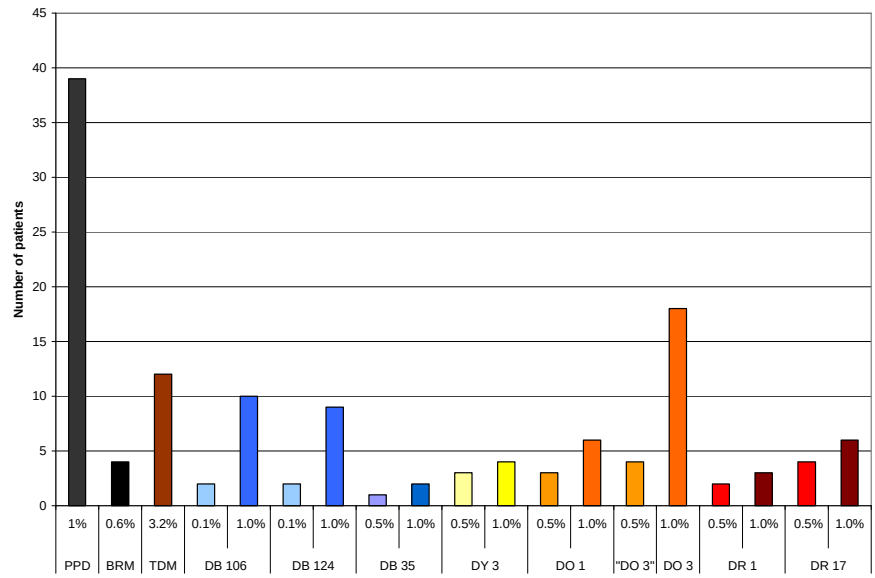


Figure 11. *Contact allergy to PPD, BRM, the TDM, and its ingredients simultaneously tested at two concentrations in 500 patients in Leuven.*

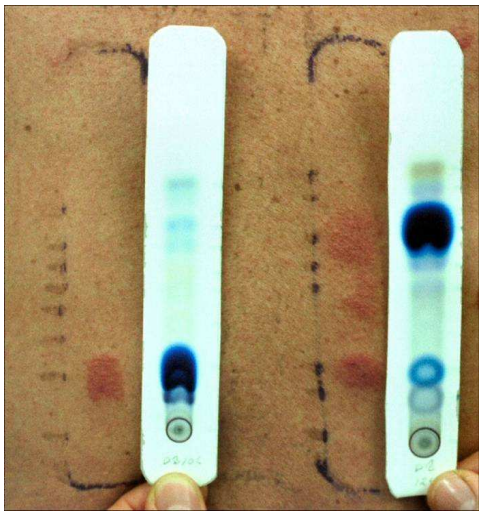


Figure 12. *Patch test reactions to DB 106 and DB 124, chromatograms A.*

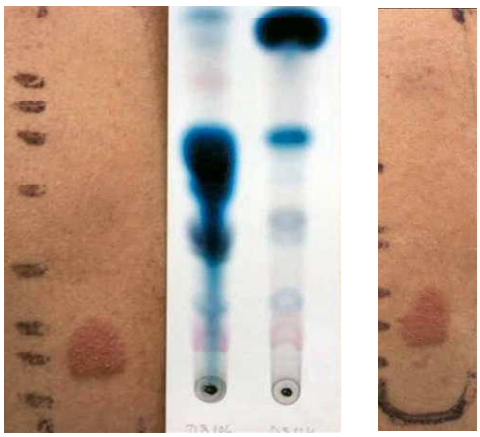


Figure 13. *Patch test reactions to DB 106 and DB 124, chromatograms B.*

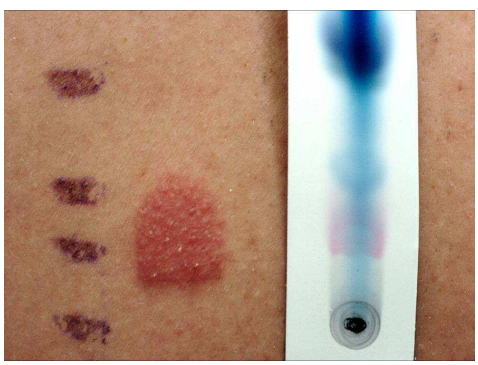


Figure 14. *A patch test reaction to the pink spot.*

4.3 Questionnaire on skin problems arising from textiles

About 18% of the patients who answered the questionnaire in Study II suspected textiles to be the possible cause of their skin problems; 20% of the Malmö patients and 16% of the patients from Leuven (P = 0.17); 22% of the females and 12% of the males (P < 0.001). In the multiple logistic regression analysis female gender, increasing age, a history of childhood eczema and contact allergy to PPD were all found to be important risk factors for self-reported textile-related skin problems (Table 11).

Table 11. *Odds ratios (OR), confidence intervals (C.I.), and P values (obtained by multiple logistic regression) for predictors of a positive answer to the question concerning self-reported textile-related skin problems. The baseline odds refers to a 40-year-old male with no childhood eczema and with negative PPD reaction.*

Factor	OR	95% C.I.	P values
Baseline odds	0.10	0.07–0.14	<0.001
Gender (female vs. male)	1.95	1.33–2.92	<0.001
Age (per 10 years)	1.14	1.02–1.28	0.022
Childhood eczema	2.26	1.49–3.40	<0.001
PPD-positive	2.14	1.01–4.31	0.039

The individual results can be found in Tables 12–14. Individual P values are given for comparisons of the answers to the questions from all patients, between females and males, and also between the patients in Leuven and Malmö. It should be noted that multiple statistical analyses may inflate the risk of false positive findings.

Contact allergy
Of the 155 patients in Study II who reported skin problems arising from textiles, 3.2% were allergic to TDM, compared to 1.7% who had no skin problems (P = 0.21). 7.7% of the patients reporting skin problems were allergic to PPD, compared to 4.0% of those reporting no intolerance to textiles (P = 0.057). BRM allergy was rare and no association with skin problems was found (Tables 12 and 13).

Atopic constitution
Of the patients who answered the questionnaire, 22% had had eczema as a child; 25% of the patients in Malmö compared with 18% of the Leuven patients (P = 0.014) (Table 12); 27% females and 14% males (P < 0.001) (Table 13).

The use of hair dyes, and temporary "tourist" tattoos

The patients in Malmö had dyed their hair more often than the Leuven patients (61% vs. 44%, P< 0.001) (Table 12). About 75% of the females and 20% of the males who answered the questionnaire had used hair dye (P < 0.001) (Table 13). No significant relation was seen between skin problems resulting from textiles and contact allergy to the TDM or PPD in the patients who had used hair dyes, but a tendency was seen for BRM-positive patients not to have used hair dyes (P = 0.092) and to have had temporary "black henna" tattoos (P = 0.087) (Tables 12 and 13).

Work in the production of textiles and handling finished textiles

Less than 2% of the patients who answered the questionnaire had worked in textile production. No gender difference or relation to contact allergy to the TDM, PPD, or BRM was found. Ten percent of the patients had worked with finished textiles, 13% in Malmö compared to 5% in Leuven (P < 0.001) (Table 12); 14% of the females compared to 4% of the males (P < 0.001) (Table 13). The Malmö patients who had worked with finished textiles reacted significantly more often to PPD than the patients in Malmö who reported not having had this occupation (P = 0.030) (Table 12).

Textile materials

The patients reported synthetic materials to be the most common cause of their skin problems from textiles, followed by wool, cotton and silk (Table 14). Females reacted to wool significantly more often than males (P = 0.014), while men reacted more often to cotton (P = 0.053). A significant association was also seen in females regarding contact allergy to PPD and self-reported skin problems arising from synthetic materials (P = 0.036).

Location of dermatitis

In Study I, the sites of dermatitis in the TDM-positive patients were compared with the skin manifestations reported by all patch tested patients. The most common sites of dermatitis in TDM-positive women were the hands (71%), followed by the face (54%) and arms (36%); in TDM-positive men the hands and arms (46% each), followed by the face (32%). In the TDM-positive women overrepresentation of axillary dermatitis was the only statistically significant association noted (P = 0.036). No investigation was made into possible dermatitis in PPD-positive and BRM-positive patients in this study. In Study II, however, the patients who reported skin problems due to textiles most frequently mentioned the legs, followed by the trunk, arms, neck, and the areas around the armpits and groin as the most commonly involved skin sites, but no association was found between textile-related skin problems on a particular body area and contact allergy to TDM, PPD, BRM or formaldehyde.

Table 12. *Distribution of answers to the questionnaire in Study II, given by the patients who participated in the questionnaire in Leuven (L) and Malmö (Mö), according to department and positive patch test results. P values < 0.05 are given in bold type.*

	Answers to the questionnaire						P values			
	Yes n, (%)			No/don´t know n, (%)			Positive vs. negative answers			Positive answers
	Leuven	Malmö	L+Mö	Leuven	Malmö	L+Mö	Leuven	Malmö	L+Mö	Leuven/Malmö
Have you ever had a rash/itch from textiles?										
All (857)	77 (20.1)	78 (16.5)	155 (18.1)	306 (79.9)	396 (83.5)	702 (81.9)				0.17
TDM-pos.	4 (5.2)	1 (1.3)	5 (3.2)	6 (2.0)	6 (1.5)	12 (1.7)	0.12	> 0.3	0.21	> 0.3
PPD-pos.	9 (11.7)	3 (3.8)	12 (7.7)	21 (6.9)	7 (1.8)	28 (4.0)	0.16	0.22	0.057	> 0.3
BRM-pos.	0	1 (1.3)	1 (0.6)	5 (1.6)	3 (0.8)	8 (1.1)	> 0.3	> 0.3	>0.3	> 0.3
Have you had ezema as a child?										
All (852)	67 (17.7)	117 (24.7)	184 (21.6)	311 (82.3)	357 (75.3)	668 (78.4)				**0.014**
TDM-pos.	3 (4.5)	1 (0.9)	4 (2.2)	6 (1.9)	6 (1.7)	12 (1.8)	0.20	> 0.3	>0.3	> 0.3
PPD-pos.	4 (6.0)	3 (2.6)	7(3.8)	25 (8.0)	7 (2.0)	32(4.8)	> 0.3	> 0.3	>0.3	> 0.3
BRM-pos.	1 (1.5)	0	1(0.5)	3 (1.0)	4 (1.1)	7(1.1)	> 0.3	> 0.3	>0.3	> 0.3
Have you dyed your hair?										
All (852)	164 (43.5)	288 (60.6)	452 (53.1)	213 (56.5)	187 (39.4)	400 (46.9)				**< 0.001**
TDM-pos.	6 (3.7)	3 (1.0)	9 (2.0)	4 (1.9)	4 (2.1)	8 (2.0)	> 0.3	> 0.3	>0.3	> 0.3
PPD-pos.	15 (9.1)	8 (2.8)	23 (5.1)	15 (7.0)	2 (1.1)	17 (4.3)	> 0.3	> 0.3	>0.3	0.145
BRM-pos.	0	2 (0.7)	2 (0.4)	5 (2.3)	2 (1.1)	7 (1.8)	0.071	> 0.3	0.092	0.17
Have you had a tourist tattoo made on you?										
All (850)	26 (6.8)	22 (4.7)	48 (5.6)	355 (93.2)	447 (95.3)	802 (94.4)				0.18
TDM-pos.	1 (3.8)	1 (4.5)	2 (4.2)	9 (2.5)	5 (1.1)	14 (1.7)	> 0.3	0.25	0.28	>0.3
PPD-pos.	2 (7.7)	2 (9.1)	4 (8.3)	28 (7.9)	8 (1.8)	36 (4.5)	> 0.3	0.075	0.28	0.256
BRM-pos.	2 (7.7)	0	2 (4.2)	3 (0.8)	4 (0.9)	7 (0.9)	**0.039**	> 0.3	0.087	> 0.3
Do you work, or have you worked with textiles										
a) in production of textiles/dyes?										
All (849)	5 (1.3)	10 (2.1)	15 (1.8)	370 (98.7)	464 (97.9)	834 (98.2)				>0.3
TDM-pos.	0	0	0	9 (2.4)	7 (1.5)	16 (1.9)	> 0.3	> 0.3	>0.3	
PPD-pos.	1 (20.0)	0	1 (6.7)	28 (7.6)	10 (2.2)	38 (4.6)	> 0.3	> 0.3	>0.3	> 0.3
BRM-pos.	0	0	0	4 (1.1)	4 (0.9)	8 (1.0)	> 0.3	> 0.3	>0.3	
b) where finished textiles are handled?										
All (845)	20 (5.4)	62 (13.1)	82 (9.7)	350 (94.6)	413 (86.9)	763 (90.3)				**<0.001**
TDM-pos.	0	1 (1.6)	1(1.2)	9 (2.6)	6 (1.5)	15 (2.0)	> 0.3	> 0.3	>0.3	> 0.3
PPD-pos.	1 (5.0)	4 (6.5)	5 (6.1)	26 (7.4)	6 (1.5)	32 (4.2)	> 0.3	**0.030**	>0.3	**0.014**
BRM-pos.	0	0	0	4 (1.1)	4 (1.0)	8 (1.0)	> 0.3	> 0.3	>0.3	

(n), number of patients answering each question; pos., positive.

Table 13. *Distribution of answers to the questionnaire in Study II, according to sex and positive patch test results. P values < 0.05 are marked in bold type.*

	Answers to the questionnaire						P values			
	Yes n, (%)			No/don´t know n, (%)			Positive vs. negative answers			Positive answers
	W	M	W+M	W	M	W+M	W	M	W+M	W/M
Have you ever had a rash/itch of textile?										
All (857)	112 (22.5)	43 (11.9)	155 (18.1)	385 (77.5)	317 (88.1)	702 (81.9)				**<0.001**
TDM-pos.	3 (2.7)	2 (4.7)	5 (3.2)	8 (2.1)	4 (1.3)	12 (1.7)	>0.3	0.15	0.21	
PPD-pos.	9 (8.1)	3 (7.0)	12 (7.7)	17 (4.4)	11 (3.5)	28 (4.0)	0.15	0.23	0.057	
BRM pos.	0	1 (2.3)	1 (0.6)	1 (0.3)	7 (2.2)	8 (1.1)	>0.3	>0.3	>0.3	
Have you had ezema as a child?										
All (852)	133 (27.0)	51 (14.2)	184 (21.6)	359 (73.0)	309 (85.8)	668 (78.4)				**<0.001**
TDM-pos.	3 (2.3)	1 (2.0)	4 (2.2)	7 (1.9)	5 (1.6)	12 (1.8)	>0.3	>0.3	>0.3	
PPD-pos.	5 (3.8)	2(3.9)	7(3.8)	20 (5.6)	12(3.9)	32(4.8)	>0.3	>0.3	>0.3	
BRM-pos.	0	1(2.0)	1(0.5)	0	7(2.3)	7(1.1)	-	>0.3	>0.3	
Have you coloured your hair?										
All (852)	379 (76.7)	73 (20.4)	452 (53.1)	115 (23.3)	285 (79.6)	400 (46.9)				**<0.001**
TDM-pos.	7 (1.8)	2 (2.7)	9 (2.0)	4 (3.5)	4 (1.4)	8 (2.0)	0.29	>0.3	>0.3	
PPD-pos.	21 (5.5)	2 (2.7)	23 (5.1)	5 (4.3)	12 (4.2)	17 (4.3)	>0.3	>0.3	>0.3	
BRM-pos.	0	2 (2.7)	2 (0.4)	1 (0.9)	6 (2.1)	7 (1.8)	0.23	>0.3	0.092	
Have you had a tourist tattoo made on you?										
All (850)	30 (6.1)	18 (5.0)	48 (5.6)	462 (93.9)	340 (95.0)	802 (94.4)				>0.3
TDM-pos.	1 (3.3)	1 (5.6)	2 (4.2)	9 (1.9)	5 (1.5)	14 (1.7)	>0.3	0.27	0.28	
PPD-pos.	2 (6.7)	2 (11.1)	4 (8.3)	24 (5.2)	12 (3.5)	36 (4.5)	>0.3	0.15	0.28	
BRM-pos.	1 (3.3)	1 (5.6)	2 (4.2)	0	7 (2.1)	7 (0.9)	0.061	>0.3	0.087	
Do you work or have you worked with textiles										
a) production of textile/dyes?										
All (849)	8 (1.6)	7 (2.0)	15 (1.8)	484 (98.4)	350 (98.0)	834 (98.2)				>0.3
TDM-pos.	0	0	0	10 (2.1)	6 (1.7)	16 (1.9)	>0.3	>0.3	>0.3	
PPD-pos.	1 (12.5)	0	1 (6.7)	24 (5.0)	14 (4.0)	38 (4.6)	>0.3	>0.3	>0.3	
BRM-pos.	0	0	0	0	8 (2.3)	8 (1.0)	-	>0.3	>0.3	
b) were finished textile is handled?										
All (845)	68 (13.9)	14 (3.9)	82 (9.7)	422 (86.1)	341 (96.1)	763 (90.3)				**<0.001**
TDM-pos.	0	1(7.1)	1(1.2)	10 (2.4)	5 (1.5)	15 (2.0)	>0.3	0.22	>0.3	
PPD-pos.	3 (4.4)	2 (14.3)	5 (6.1)	21 (5.0)	11 (3.2)	32 (4.2)	>0.3	0.088	>0.3	
BRM-pos.	0	0	0	0	8 (2.3)	8 (1.0)	-	>0.3	>0.3	

M, men; (n), number of patients answering each question; pos., positive; W, women.

Table 14. *Distribution of answers to the question "which type of textile material?" in Study II, given by the patients who suspected textiles to be the cause of their skin problems, according to sex and patch test results. P values < 0.05 are marked in bold type.*

	Answers to the questionnaire						P values			
	Yes, n (%)			No/don't know, n (%)			Positive vs. negative answers			Positive answers
	W	M	W+M	W	M	W+M	W	M	W+M	W/M
Which type of textile?										
Wool (156)	46 (40.7)	8 (18.6)	54 (34.6)	67 (59.3)	35 (81.4)	102 (65.4)				**0.014**
TDM-pos.	2 (4.3)	1 (12.5)	3 (5.6)	1 (1.5)	1 (2.9)	2 (2.0)	>0.3	>0.3	>0.3	
PPD-pos.	3 (6.5)	0	3 (5.6)	6 (9.0)	3 (8.6)	9 (8.8)	>0.3	>0.3	>0.3	
BRM-pos.	0	1 (12.5)	1 (1.9)	0	0	0	-	0.19	>0.3	
Cotton (156)	20(17.7)	14 (32.6)	34 (21.8)	93 (82.3)	29 (67.4)	122 (78.2)				0.053
TDM-pos.	0	0	0	3 (3.2)	2 (6.9)	5 (4.1)	>0.3	>0.3	>0.3	
PPD-pos.	2 (10.0)	0	2 (5.9)	7 (7.5)	3 (10.3)	10 (8.2)	>0.3	>0.3	>0.3	
BRM-pos.	0	0	0	0	1 (3.4)	1 (0.8)	-	>0.3	>0.3	
Synthetic (155)	60(53.6)	29(67.4)	89(57.4)	52 (46.4)	14 (32.6)	66 (42.6)				0.15
TDM-pos.	2 (3.3)	1 (3.4)	3 (3.4)	1 (1.9)	1 (7.1)	2 (3.0)	>0.3	>0.3	>0.3	
PPD-pos.	8 (13.3)	2 (6.9)	10 (11.2)	1 (1.9)	1 (7.1)	2 (3.0)	**0.036**	>0.3	0.072	
BRM-pos.	0	1 (3.4)	1 (1.1)	0	0	0	-	>0.3	>0.3	
Silk (156)	7(6.2)	3(7.0)	10(6.4)	106 (93.8)	40 (93.0)	146 (93.6)				>0.3
TDM-pos.	0	0	0	3 (2.8)	2 (5.0)	5 (3.4)	>0.3	>0.3	>0.3	
PPD-pos.	0	0	0	9 (8.5)	3 (7.5)	12 (8.2)	>0.3	>0.3	>0.3	
BRM-pos.	0	0	0	0	1 (2.5)	1 (0.7)	-	>0.3	>0.3	

M, men; (n), number of patients answering each question; pos., positive; W, women.

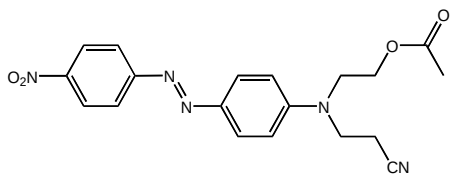
4.4 Chemical investigations

4.4.1 High performance liquid chromatography

A total of 107 patch test preparations were investigated in Study IV. The results of the HPLC analyses are presented in Table 15. The mean concentration of the patch test preparations labelled 1.0 or 1% varied from 0.25% for DB 124 to 0.68% for DO 3, when excluding 4 test preparations in which DO 3 could not be detected (detection limit 0.009%). HPLC analyses of these 4 divergent patch test preparations showed that they contained another orange dye, and MS investigations, also performed at our department in Malmö, revealed that they contained DO 31 instead (Figure 15). Only 2 patch test preparations, both labelled DR 1, contained the stated concentration. No correlation was found between the expiry date and the concentration of the disperse dyes.

Furthermore, although 7 of the 8 investigated disperse dyes were chemically defined, the HPLC analyses of the patch test preparations gave several peaks, demonstrating the presence of impurities. The HPLC analyses also demonstrated that the DB 106

patch test preparations labelled 1.0% contained DB 124, varying from 0.002 to 0.0004%, and the preparations labelled DB 124 1.0% contained DB 106, varying from 0.06 to 0.005%. Investigations of the separate fractions of DB 35 with MS identified at least six substances, four of which were anthraquinones. The HPLC analyses of DB 35, showing several peaks, and DB 106, showing one main peak, can be seen in Figure 16**.**



CAS: 68391-42-4
CI:111135
MW: 381
log $P_{o/w}$: 3.12

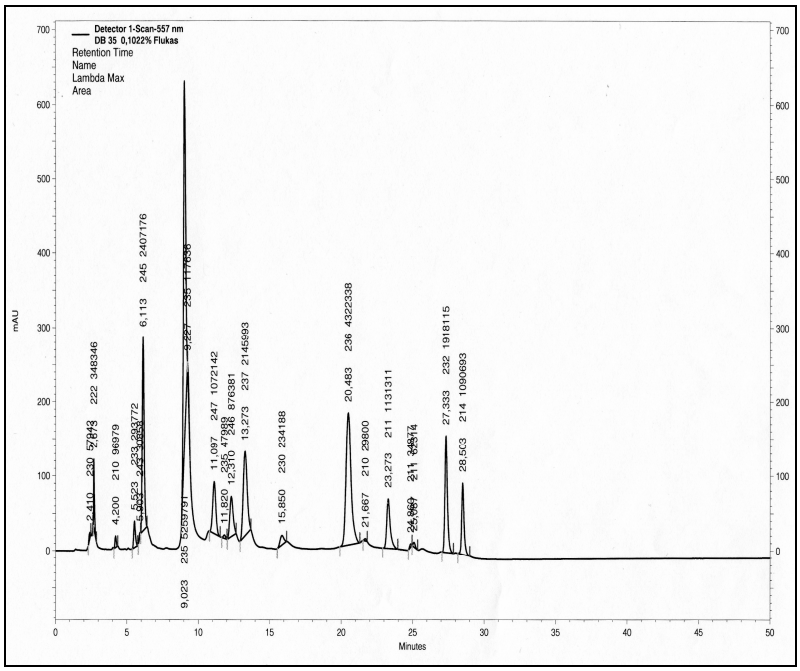
Figure 15. *The chemical structure of Disperse Orange 31, including the Chemical Abstract Service (CAS) and Colour Index (CI) number, and the molecular weight (MW) and value of log P*o/w**.**

4.4.2 Thin-layer chromatography

The thin-layer chromatograms of the acetone solutions from the extracts of the 107 patch test preparations used in Study IV showed different patterns, varying from 11 spots in the DB 124 preparations to 1 defined spot in 11 of the 12 DO 1 patch test preparations (Figure 17). The DB 35 preparations, which had many peaks in the HPLC analyses (Figure 16), all lacked a main spot, but showed at least 5 equally intense spots on the thin-layer chromatograms (Figure 18 A). The oldest preparations showed the same TLC pattern as preparations that had not expired.

Small amounts of DB 124 in the DB 106 preparations, and vice versa, were demonstrated by the HLPC analyses and also observed on the relevant thin-layer chromatograms (Figure 18 B). Pink spots were seen on the TLC plates from 10/12 DB 106 preparations and 13/14 DB 124 preparations (Figure 18 B). The corresponding pattern with defined pink spots was also seen on DB 106 and DB 124 chromatograms (B) used for TLC testing in Study V.

The 4 patch test preparations in which DO 3 could not be detected when analysed with HPLC ("DO 3" preparations) also showed divergent patterns on the chromatograms, with other spots, another main spot, and lacking the spot corresponding to the main spot of the remaining 11 DO 3 preparations. Patch test preparations representing the two variants of preparations labelled DO 3 can be seen in Figure 18 C.



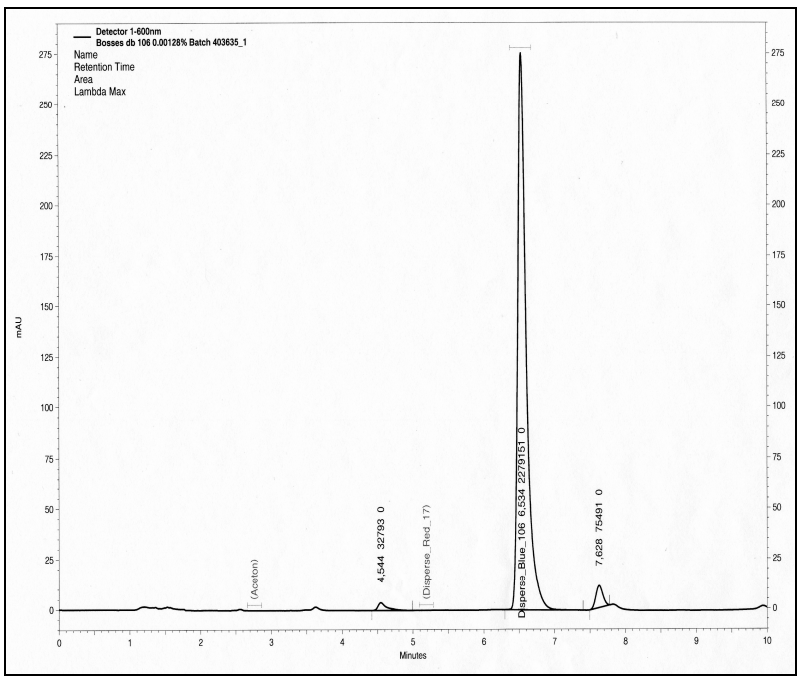


Figure 16. *HPLC analysis of DB 35 (above) and DB 106 (below).*

Table 15. *The disperse dye patch test preparations used by the various departments, with the suppliers, and the stated and detected concentrations in each test preparation analysed with HPLC in Study IV.*

	Disperse Blue 106					Disperse Blue 124				
Dept.	**Supplier**	**Stated # conc.** (% w/w)	**Detected conc.** (% w/w)	**C.V.** (%)	**Expired**	**Supplier**	**Stated conc.** (% w/w)	**Detected conc.** (% w/w)	**C.V.** (%)	**Expired**
A	I	1.0	0.30	18.8		I	1.0	0.24	25.3	
B	I	1.0	0.32	14.6	*	I	1.0	0.25	15.4	**
C	nd	0.1	0.04•	14.4	**	I	1.0	0.23	6.6	**
D	I	1.0	0.32	26.5		I	1.0	0.23	[]	
E	I	1.0	0.27	22.5	*	I	1.0	0.25	6.9	*
F	I	nd	0.33	9.8		III	nd	0.18	6.5	
G	I	1.0	0.36	11.1	*	I	1.0	0.26	13.3	*
H	I	1.0	0.34	10.2	*	I	1.0	0.23	11.5	**
I	I	1.0	0.33	12.4	*	I	1.0	0.34	[]	*
J	I	1.0	0.32	26.9	*	I	1.0	0.25	14.4	*
K	I	1.0	0.13	22.8	*	I	1.0	0.24	8.8	*
L	I	1.0	0.33	18.4	*	I	1.0	0.27	7.4	**
M	¤	1.0	0.29	27.4		¤	1.0	0.24	7.2	
	-	-	-	-	-	IV	1	0.26	4.4	
Mean conc. of detected dye			**0.30**					**0.25**		
	Disperse Blue 35					**Disperse Yellow 3**				
Dept.	**Supplier**	**Stated conc.** (% w/w)	**Detected conc.** (% w/w)	**C.V.** (%)	**Expired**	**Supplier**	**Stated conc.** (% w/w)	**Detected conc.** (% w/w)	**C.V.** (%)	**Expired**
A	I	1.0	na			I	1.0	0.50	18.0	
B	I	1	na		*	I	1	0.39	6.4	*
C	I	1.0	na		**	III	1	0.44	4.8	**
D	I	1.0	na		**	I	1.0	0.43	0	
E	I	1	na		*	I	1.0	0.41	5.1	*
F	-	-	na		-	III	nd	0.46	12.1	
G	I	1.0	na		**	II	1	0.47	7.7	*
H	I	1.0	na		*	II	1	0.40	5.7	*
I	I	1.0	na		*	I	1.0	0.49	[]	*
J	I	1.0	na		*	I	1.0	0.27	[]	*
K	I	1.0	na		*	I	1.0	0.40	10.9	*
L	I	1.0	na		*	I	1.0	0.43	7.0	*
M	¤	1.0	na			¤	1.0	0.61	43.5	
	-	-			-	IV	1	0.48	14.0	
Mean conc. of detected dye								**0.44**		

		Disperse Orange 1					Disperse Orange 3			
Dept.	**Supplier**	**Stated conc.** (% w/w)	**Detected conc.** (% w/w)	**C.V.** (%)	**Expired**	**Supplier**	**Stated conc.** (% w/w)	**Detected conc.** (% w/w)	**C.V.** (%)	**Expired**
A	I	1.0	0.46	35.9		I	1.0	0.88	4.0	
B	I	1	0.39	21.9	*	I	1	0.25	4.0	*
C	I	1.0	0.36	13.6	**	I	1.0	0.77	1.5	
D:a	I	1.0	0.34	16.4		I	1.0	0.85	11.2	
D:b	-	-	-	-	-	II	1	0.80	5.6	
E	I	1.0	0.36	20.3	*	I	1.0	<0.009		*
F	-	-	-	-	-	III	nd	0.38	4.1	**
G	I	1.0	0.35	24.7	*	II	1	0.79	8.2	*
H	I	1.0	0.42	17.7	*	II	1	0.92	3.8	*
I	I	1.0	0.57	[]	*	I	1.0	<0.009		*
J	I	1.0	0.40	13.2	*	I	1.0	<0.009		*
K	I	1.0	0.36	24.2	*	I	1.0	0.93	4.3	
L	I	1.0	0.39	14.9	*	I	1.0	0.61	0.9	
M	¤	1.0	0.37	7.2		¤	1.0	<0.009		
	-	-	-	-	-	IV	1	0.34	4.5	
Mean conc. of detected dye			**0.40**					**0.68**		

		Disperse Red 1					Disperse Red 17			
Dept.	**Supplier**	**Stated conc.** (% w/w)	**Detected conc.** (% w/w)	**C.V.** (%)	**Expired**	**Supplier**	**Stated conc.** (% w/w)	**Detected conc.** (% w/w)	**C.V.** (%)	**Expired**
A	I	1.0	0.37	31.7		I	1.0	0.35	26.2	
B	I	1	0.39	10.3	*	I	1	0.38	2.6	*
C	I	1.0	0.35	[]	**	I	1.0	0.32	3.6	**
D	I	1.0	0.45	[]		I	1.0	0.33	6.1	
E	I	1.0	0.37	15.0	*	I	1	0.28	6.2	*
F	III	nd	0.39	11.8		-	-	-	-	-
G	II	1	1.01	15.3	*	II	1	0.40	6.2	*
H	II	1	1.03	17.5	*	II	1	0.34	9.1	*
I	I	1.0	0.43	[]	*	I	1.0	0.31	27.9	
J	I	1.0	0.41	21.3	*	I	1.0	0.39	11.8	*
K	I	1.0	0.42	12.4	*	I	1.0	0.38	5.3	*
L	I	1.0	0.39	12.0	*	I	1.0	0.33	10.9	*
M	¤	1.0	0.38	5.4		¤	1.0	0.27	18.4	
	IV	1	0.47	11.3		IV	1	0.50	8.7	
Mean conc. of detected dye			**0.49**					**0.35**		

Conc., concentration; C.V., coefficient of variation for the triplicate samples; na, not applicable; nd, not declared. # Stated concentration according to the labelling on the syringes. • Test preparation excluded when mean concentration was calculated. [] Syringes contained too little test material for triplicate analysis. ¤ Test preparation made at the department in Malmö * Expiry date passed at arrival at the department. ** Expiry date passed during the investigative period.

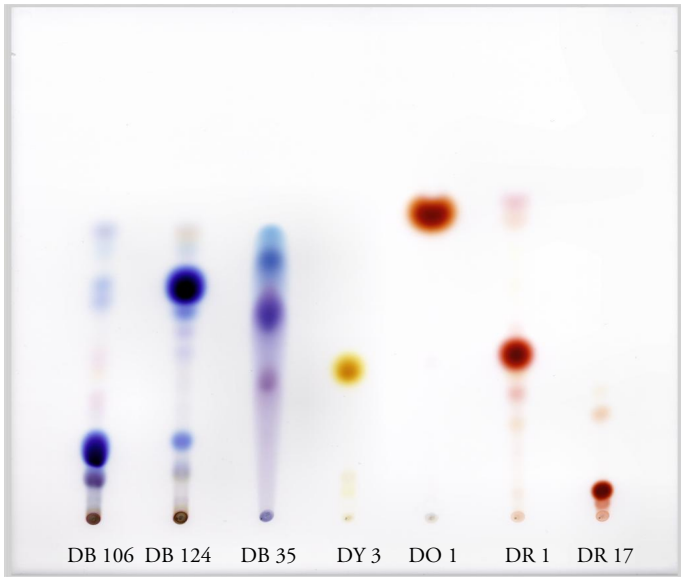


Figure 17. *Thin-layer chromatograms of 7 of the 8 dyes investigated. The chromatograms of DO 3 and "DO 3" can be seen below.*

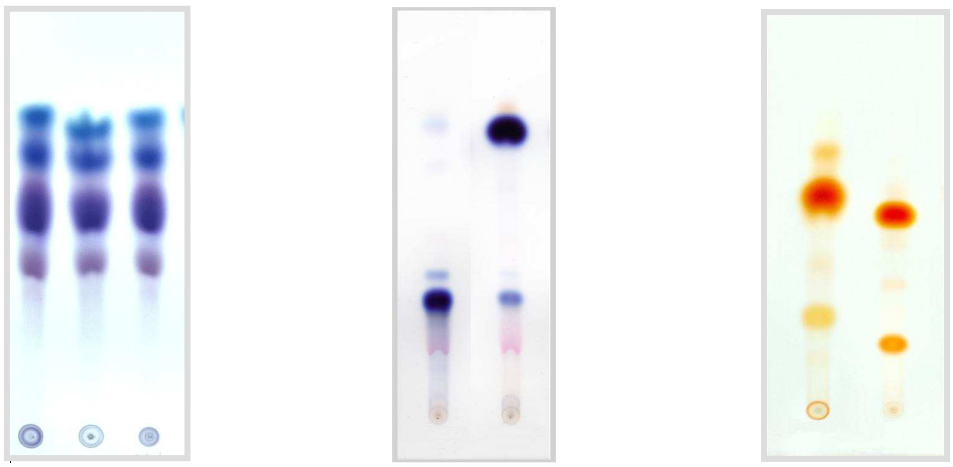


Figure 18. *Thin-layer chromatograms of DB 35 (A), DB 106 and DB 124 (B), and 2 patch test preparations labelled DO 3 (C).*

5. DISCUSSION

Contact allergy to textile dyes has been documented in prevalence studies among consecutively patch-tested dermatitis patients, especially in southern Europe (93,122-124,128). Patients have been diagnosed as having textile dermatitis and contact allergy to disperse dyes at our department in Malmö, but there have probably also been cases where contact allergy to disperse dyes has been missed, due to the fact that disperse dyes are not included in the baseline series, and because the clinical picture does not always indicate textile dermatitis. Prevalence studies in consecutively patch-tested patients have not been conducted in the Scandinavian countries until now.

5.1 Patch testing with a textile dye mix in the baseline series

In the first study, the prevalence of contact allergy to a TDM and to the 8 disperse dyes in the mix, i.e. DB 106, DB 124, DB 35, DY 3, DO 1, DO 3, DR 1, and DR 17, included in the baseline patch test series in Malmö, from 1999 to 2003 was investigated (Paper I). The results, showing contact allergy to the mix in 1.5% of the patch-tested patients, was comparable to that found in other studies from southern Europe (30,128). The European Society of Contact Dermatitis recommends a sensitizer to be included in the baseline series when screening patients with suspected contact dermatitis results in a contact allergy rate exceeding 0.5-1% (143). However, no reports of such a TDM were found in the literature, although a mix with an identical composition has been used for patch testing in Portugal (Dr Francisco Brandão, Department of Dermatology, Hospital Garcia de Orta, Almada, Portugal, personal communication, 1998). The results from patch testing with the TDM in Malmö and in Leuven from 2004 to 2005 (Paper III) confirmed the results from the first study, as 2.0% of the patients reacted to the mix. No statistically significant differences in the prevalence of contact allergy to the TDM were found between the two studies (I, III), or between the 2 departments included in Study III.

Patch testing with a mix of dyes saves space and time, but further studies were necessary to determine whether the TDM could be used for screening of contact allergy to the 8 disperse dyes included in the mix. According to the results of Study III, the TDM can be used for screening of patients with contact allergy to the ingredients in the mix, as the mix detected as many patients with contact allergy as simultaneous patch testing with the 8 ingredients separately, at the concentrations used in the mix. However, 9 of the 34 patients who were allergic to at least 1 ingredient tested at the concentration used in the mix would have been missed if only patch tested with the TDM (Study III, Figures 2 and 3). Simultaneous testing with 6 of the 8 ingredients in the TDM would have been necessary to identify these 9 patients.

Of the TDM-positive patients, 36% in Study I and 29% in Study III tested negatively to the separate ingredients at the same concentrations as those used in the mix. One possible explanation of why the ingredient testing was negative in some TDM-positive patients could be that the penetration of the skin by the ingredients in the mix was higher when tested together in a mix than that of the separate ingredients. Other explanations could be a compound allergy caused by additive or synergistic effects of the different substances, as has been demonstrated when testing other mixes such as fragrance mix (144,145). The additive and/or synergistic effect implies that single allergens patch tested below the threshold for a patch-test reaction could give a positive test response when tested in combination, as the reaction to the mixture of allergens will be the sum or above the sum, respectively, of the individual components (146).

5.2 Patch testing with the ingredients in the textile dye mix

In this section, the results of testing the ingredients at the concentrations used in the mix in all 1780 patients (Study I), and the results of simultaneous testing at the higher concentration in the Leuven patients (Study III) will be discussed.

Disperse Orange 1

At the concentration used in the mix, 0.5% w/w (pet.), DO 1 was the most common disperse dye allergen, in both studies (Papers I & III). The intensity of the patch test reactions to DO 1 in the majority of the patients in both studies was classified as +++, which is in contrast to the response to the other dyes in the mix. The reason for this is not currently known. DO 1 is not commonly used for patch testing, and only a few studies employing this dye were found in the literature (Table 4) (10,126,127), none of which describe consecutively patch-tested patients. When the Portuguese Contact Dermatitis Group reviewed their patch test data from the last years in 2004, DO 1 was found to be one of the more common sensitizers (Dr Francisco Brandão, Almada, Portugal, personal communication, 2004). Furthermore, as can be seen in Figure 9, 56% of the 27 patients in Studies I+III who were allergic to both TDM and PPD, and more than 80% of the patients who were allergic to both TDM and BRM were also allergic to DO 1 when tested at the concentration in the mix. A highly significant association was found between contact allergy to DO 1 and PPD on the one hand, and DO 1 and BRM on the other, among the patients in Study III, for both males and females. The connection between contact allergy to DO 1 and PPD or BRM was also observed in a previous study carried out at the department in Leuven (126), and the author concluded that BRM sensitivity "...could, in some cases, indicate or perhaps even induce contact allergy to azo dyes used in textiles".

Disperse Blue 106 and Disperse Blue 124

Both DB 106 and DB 124 have been identified as strong allergens by several authors (see Section 1.3.4) (78,113,115). These results were verified in Study V as some patients patch tested with dilution series of purified DB 106 and DB 124 reacted to 0.010 µg/ml (~ 0.010 ppm), the lowest concentration tested. Furthermore, earlier studies have reported DB 106 and DB 124 to be the most common allergens among the 8 disperse dyes tested (78,124,147). However, the prevalence of allergic reactions to DB106 and DB 124 was lower than expected in both Study I and Study III. When patch tested simultaneously at a 10 times higher concentration (1.0%) in Leuven (Paper III), 5 and 4.5 times more patients were found to have contact allergy to DB 106 and DB 124, respectively, compared with patch testing with the dyes at the concentrations used in the mix (Figure 11). Different patch test concentrations have been recommended for the testing of DB 106 and DB 124. A concentration of 0.1% was recommended in a study conducted in Portugal in 1987 (27); in the textile and leather dyes series from Trolab DB 106 and DB 124 are patch tested at 0.3% (148); in the textile colours and finish series from Chemotechnique the 2 dyes are tested separately at 1.0% as well as in a DB mix of 106/124 (1.0% each) (149); and in a German study from 2007, patch testing of both dyes at 0.3% rather than 1.0% was recommended, especially in the case of DB 106 (118), because of its strong allergenic potential.

In both Study IV and in the German study (118), the mean concentrations of purified DB 106 and DB 124 in the commercial patch test preparations were only 25 to 30% of the labelled concentration when analysed with HPLC and compared with purified reference substances. This implies that when DB 106 or DB 124 preparations labelled 0.1% are used for testing, they may in reality only contain 0.025-0.030% of the chemically defined dye. Apart from the effect of patch testing with a lower concentration of the purified dye than expected, another problem may arise from different batches being used to prepare patch test solutions. The concentration of the chemically defined disperse dyes and the presence and concentrations of impurities may vary significantly between different batches, as demonstrated in Study IV and later confirmed by the study in Germany (118).

Furthermore, approximately 25% of the patients in Study V, previously regarded as being allergic to DB 106 and DB 124, were found to be allergic to impurities in the preparations, and not to the purified dyes themselves (Paper V). All these findings emphasize the importance of investigating the concentration of the purified dye as well as the content of different impurities, not only in patch test preparations containing DB 106 and DB 124, but in patch test preparations in general, to be able to determine their optimal purity, concentration and vehicle for the detection of contact allergy, without the risk of sensitizing the patient through patch testing.

Disperse Yellow 3

Dermatologists reported contact allergy to disperse dyes, especially to DY 3 and DO 3, in stockings 30 years ago (25). These dyes were also found to be present in stockings and briefs in a thin-layer chromatography investigation in 1984 (150). As mentioned above, the sensitizing potential of DY 3 was regarded as being moderate to weak in animal tests, but as a result of its frequent use in clothes, especially in stockings and tights, it has been a frequently reported contact allergen in ACD. In both Study I and Study III, DY 3 was the next most common allergen after DO 1, while in Study III it was found to be as common a contact allergen as DR 17. However, the use of DY 3 is now restricted within the EU because of its possible carcinogenic properties (69). This may lead to a decrease in the number of patients with contact allergy to DY 3 in the future.

Disperse Orange 3

In the aforementioned study from Leuven (126), 159 patients with suspected textile-related dermatitis were patch tested with 15 textile dyes, including the 8 disperse dyes, during 5 years from 1987. This testing revealed 28 patients with contact allergy to textile dyes. Although it is difficult to compare the results from this aimed testing with the results from consecutively patch-tested screening patients in Study III, a comparison demonstrates an increasing prevalence of contact allergy to DO 3 in Belgium. The results from these two studies can be seen in Table 16.

Furthermore, considering the different populations tested in the two studies, the results indicate an increasing prevalence of contact allergy to DO 1 and DR 1 among the Leuven patients. A possible explanation of the increasing prevalence of contact

Table 16. *Contact allergy to the 8 disperse dyes, tested at 1.0% w/w (pet.), in 2 different studies at the Department of Dermatology, Katholieke Universiteit in Leuven, Belgium.*

Disperse dye	Leuven 1987-1991 Aimed testing (159 patients)		Leuven 2004-2005 Screening (500 patients)	
	No. allergic	%	No. allergic	%
DB 106*	16	9.8	10	2.0
DB 124	6	3.8	9	1.8
DB 35	6	3.8	2	0.4
DY 3*	6	3.7	4	0.8
DO 1	2	1.3	6	1.2
DO 3*	5	3.0	18	3.6
DR 1	2	1.3	3	1.6
DR 17	6	3.8	6	1.2

* 164 patch-tested patients 1987-1991.

allergy to all these disperse dyes could be cross-reactivity with PPD, as PPD was a more common allergen in the patients who reacted to the disperse dyes in the Leuven patients in Study III (46.2%), than in the previous Leuven study (28.5%).

Patch testing with the authentic DO 3 would probably have given a higher prevalence of contact allergy to this dye when tested at the concentration in the mix, as simultaneous patch testing with DO 3 at twice the concentration, 1%, in Leuven gave more than 4 times as many patients with contact allergy than patch testing with "DO 3" at the concentration in the mix (Figure 11). The majority of the patients who were allergic to DO 3 at the higher concentration were also allergic to PPD. Simultaneous contact allergy to PPD and DO 3 has been described in several studies (25,72,123). PPD has long been considered to be a good screening allergen for textile dye dermatitis (71), but nowadays it is considered to be a better screening agent for contact allergy to hair dyes, than for allergy to disperse dyes (127). However, the present findings indicate that PPD is still useful in identifying patients with contact allergy to disperse dyes, especially to DO 3. This is supported by the results in Study II, where contact allergy to PPD was found to be an important risk factor for self-reported textile-related skin problems.

In contrast, none of the patients who were allergic to DB 106 or DB 124 in the first study reacted to PPD or BRM (Paper I). These results were confirmed by the results in Study III, where PPD only identified 1 of the Leuven patients who was allergic to DB 106 and DB 124 at 1.0%, but not to DO 3 at 1.0%. The same results have been reported in other studies, where simultaneous contact allergy to PPD and/or DB 106 or DB 124 were unusual (123,126,127,138,151). A different clinical pattern was seen in the two groups of patients. Hand dermatitis, occupational exposure to hair dyes and intolerance to dyes used for colouring their own hair was found in the patients with contact allergy to PPD and DO 3, while the patients who were allergic to DB 106 and DB 124 had a history of intolerance to textile garments, with a high degree of clinical relevance of the positive patch test results (123). Many studies on contact allergy to disperse dyes identify DB 106 (126), or DB 106 and DB 124, as the disperse dye(s) of greatest interest and recommend them as screening allergens for textile dye dermatitis (78,136,152). However, Seidenari et al. suggested that a combination of DB 106, DB 124 and DO 3 could be introduced in the baseline series as a screening allergen, to enable further evaluation of the extent of textile dye contact allergy (125).

The pattern of concomitant contact allergy to the TDM, PPD and BRM, shown in Figure 8, demonstrates that a high percentage of the BRM-positive patients were allergic to the TDM. Therefore, a combination of PPD and BRM, both already included in most baseline series, supplemented with one dye or mix of dyes could be used in baseline screening to identify patients with contact allergy to disperse dyes.

Simultaneous testing in Study III with a combination of PPD, BRM, and DB 106 at the concentration in the mix (0.1%), identified 71% of the patients with contact allergy to at least 1 ingredient in the mix, at the concentration used in the mix. In the 500 Leuven patients, PPD, BRM and TDM together identified 79% of the 28 patients with contact allergy to at least 1 of the 8 disperse dyes tested at 1.0% (Paper III), while patch testing with PPD, BRM and DB 106 at 1.0% identified 82% of the 28 patients. However, as mentioned above, DB 106 is recommended for patch testing at 0.3% rather than 1.0% because of its strong allergenic potential. This would probably lead to a lower frequency of patients being identified as contact allergic to the 8 disperse dyes than patch testing with the combination of PPD, BRM and DB 106 at 1.0%.

For obvious reasons, simultaneous patch testing with "DO 3"/DO 3, DB 106 and DB 124, tested at different concentrations in the baseline testing in Study III, gave the most disparate patch test results (Figure 11). DB 35, DY 3, DO 1, DR 1, and DR 17 tested at twice the concentration identified 33-100% more patients with contact allergy to the individual disperse dyes in the patients simultaneously tested at two concentrations, but most of the reactions were weak and all but one patient reacted to the TDM.

5.2.1 Cross-reactivity

Cross-sensitization is the elicitation of ACD by a substance that is chemically related to the compound that caused sensitization. Multiple contact allergy must, however, be excluded before a contact allergy can be considered as cross-sensitization (153,154). Statistically highly significant associations were seen in Study III between contact allergy to PPD and contact allergy to DO 3 at 1% w/w in the Leuven patients; between allergic reactions to PPD and to the TDM; between allergic reactions to PPD and to BRM, and also between contact allergy to the TDM and to BRM (all $P < 0.001$). Simultaneous contact allergy to PPD and DO 3 has been described in other studies, and has been suggested to be due to either cross-sensitization or to the metabolic conversion of PPD and DO 3 to a common allergen in the skin (72,123). Contact allergy to PPD may indicate that the patient has been primarily sensitized by hair dye, temporary "black henna" tattoo dye or by PPD derivatives in BRM. However, the results in Study III did not demonstrate any statistically significant association between contact allergy to PPD and having used hair dye or having had temporary tattoos (Table 13). Furthermore, some of the patients who were allergic to the TDM may initially have been sensitized to PPD and then reacted to disperse dyes due to cross-reactivity, or they may have been sensitized by exposure to a common metabolite, rather than disperse dyes in textiles. Another explanation of the simultaneous contact allergy to TDM, PPD and BRM could be a common impurity present in all the patch test preparations.

In Study III, the majority of the patients with contact allergy to DB 106 were also allergic to DB 124, and vice versa. A possible explanation of cross-reactivity, given in several studies (27,113,125) is their close chemical relation, as the only difference between these two disperse dyes is the presence of an acetate group in DB 124. Hydrolysis of the ester group in DB 124, converting this substance into DB 106 and acetic acid, a metabolic reaction that can occur in the skin (155), could be another explanation of the clinically observed concomitant reactivity. The chemical investigations of DB 106 and DB 124 in Study IV revealed that both dyes contained a low amount of the other, seen as additional spots on the chromatograms and as peaks in the HPLC analyses. However, the highest possible concentration of DB 124 in the preparations of DB 106 labelled 1.0% was 0.002%, and the corresponding concentration of DB 106 in the DB 124 preparations was 0.06%. As the reactivity to the dilution series of commercial and purified DB 106 and DB 124 in Study V was the same for the individual patients this could not be the reason for the corresponding test results.

It is important to emphasize that it is impossible to draw any firm conclusions regarding cross-reactivity between different chemicals from the clinical pattern of concomitant contact allergies to chemically related patch test preparations, unless patch testing with purified preparations and animal tests (guinea-pig tests) have been performed. This assertion was verified in Study V, when some of the patients, previously regarded as being allergic to DB 106 and DB 124, reacted to impurities in the preparations, but not to the purified dyes.

5.3 Clinical relevance of a positive patch test

5.3.1 Nature of patch test reactions

Before the clinical relevance of a positive patch test reaction is discussed, the nature of positive reactions must be assessed. For test preparations commonly used in baseline series, the distinction between allergic and irritant reactions is not usually a problem, but differentiation may be more difficult regarding test preparations not so commonly used. In these studies, it was sometimes hard to differentiate a weak positive reaction from a negative reaction (140), because of the dyeing of the skin. This will lead to the risk of overdiagnosing, especially regarding DR dyes. Patch test reactions to dilution series of the suspected sensitizer and negative patch test reactions in control patients can be used to differentiate between allergic and irritant reactions (154). Patch testing with dilution series of both commercial and purified DB 106 and DB 124 was performed in Study V, but no patch testing with dilution series was carried out with the remaining disperse dyes in this research project.

Furthermore, the reading on day 7 in Study I revealed 4 more patients (8%) with positive reactions to the TDM. In Study III the second reading in Malmö gave 3 more patients (13%) with contact allergy to the TDM; while 1 of the 21 patients with positive reactions to the commercial and purified DB 106 and DB 124 patch test preparations was only detected at the reading on day 7 in Study V. These results are supported by other studies, reporting that patch test reactions to DB 106 and DB 124 are occasionally delayed up to 7 – 10 days (78). Late reactions to DO 1 and DO 3 tested at 1% (pet.) on day 10 or later have also been reported (156). In Studies I, II/III and V no late reactions were registered after day 7 in the participating patients, but the importance of at least a second reading on day 7 must be emphasized (89,157,158).

5.3.2 Exposure

Individual patients with contact allergy to disperse dyes are regularly revealed by testing with commercially available textile patch test screening series. On the other hand, there is little knowledge of the clinical relevance of positive patch tests to disperse dyes. To estimate the risk of an allergen, both the sensitization potential and the exposure to the allergen must be considered. A positive patch test reaction does not necessarily imply that the demonstrated contact allergy to the disperse dye is clinically relevant. The connection between skin problems, contact allergy and exposure to the sensitizer must be scrutinized for each individual patient. Hatch et al. (159) reported that disperse dyes to which the patients were allergic were only infrequently identified in the fabrics suspected to be the cause of their skin lesions. However, during recent years, patients with clinically relevant contact allergies to disperse dyes have been regularly diagnosed at our clinic, especially to DB 106 and DB 124. Although the European textile industry is now aware of the risk of using some disperse dyes, other dyes with related chemical and allergenic properties may still be in use. Moreover, the larger part of the textile dyeing industry is located in non-European countries today. Their products are sold to both local inhabitants and tourists, and many are exported to other countries. Consequently, it is important to control textiles produced both in European and non-European countries to identify the textile dyes currently being used. Furthermore, people may wear old clothes, and it has become popular to buy and use second-hand clothes and other textiles. We must therefore be aware of the possibility of textile-related contact allergy, in patients exhibiting skin symptoms for which there is no clear cause.

Moreover, it was found that some of the patients only reacted to impurities in the patch test preparations. It is important to investigate whether the substance(s) to which the patients reacted can be found in the disperse dyes presently used for dyeing clothes and other textiles, as well as in non-textile products, as these impurities could cause sensitization and the elicitation of ACD.

Although the questionnaire in Study II contained several questions on possible exposure to disperse dyes, this was not an epidemiological investigation aimed at determining the true prevalence of textile dermatitis. The questionnaire was used to elucidate a possible connection between self-reported textile-related skin problems and contact allergies to a TDM, PPD, BRM and formaldehyde, and various risk factors.

Contact allergy to PPD was found to be an independent risk factor for skin problems arising from textiles. There was an indication that contact allergy to the TDM, with the composition used here, was a risk factor for textile-related skin problems, but this was not proven statistically. One possible explanation of the results may be the low prevalence of patients with contact allergy to the disperse dyes tested. More reactors may have been found by patch testing with the 8 disperse dyes at higher concentrations in the mix. However, it could be that the disperse dyes used in the mix are not the best screening dyes for detecting clinically relevant textile-related skin problems caused by contact allergy to disperse dyes today, or that the self-reported skin problems also reflected irritant reactions to clothes and other textiles (3,4).

In Study II there was a tendency for patients with occupational exposure, working in the production or handling of finished textiles, to have more textile-related problems than patients who had not reported having had these occupations. However, no statistically significant association was found between these occupations and contact allergy to TDM, PPD or BRM, although PPD-positive patients working with finished textiles in Malmö more often reported textile-related skin problems than the corresponding PPD-negative Malmö patients ($P = 0.030$). Several studies have shown that ACD from disperse dyes mainly affects the hands in occupationally exposed patients (124,138), but no such relation was found in this study, perhaps due to too low a frequency of patients with occupational exposure and contact allergy to the TDM.

Location of textile-related dermatitis

In Study I the most frequently involved skin sites, both in the TDM-positive patients and in all dermatitis patients tested, were the hands, arms, and face. The sites of dermatitis in the TDM-positive patients are compared to those of all tested patients in Study I (Paper I, Figure 6). The statistically significant overrepresentation of axillary dermatitis in TDM-positive women in Study I was supported by another study (125), where involvement of skin folds was seen in 27% of disperse dye-positive women, mainly women with contact allergy to DB 106 and DB 124. However, no correlation was found between the location of dermatitis and contact allergy to separate disperse dyes in the individual patients in this work.

5.4 Differences in patch test results Leuven vs. Malmö

Contact allergy to PPD was significantly more frequent in the Belgian patients than in the Swedish patients (7.8% vs. 2.4%; $P < 0.001$, Figure 8), whereas no statistically significant differences were seen between the numbers of patients who reacted to the TDM, the ingredients in the TDM tested at the concentrations used in the mix, or BRM (Study III) at the 2 departments. Allergic reactions to PPD were also more common in Leuven than in a multicentre study including nine European countries, where 3.1% (C.I. 95% 2.7-3.3) of the patients were found to be allergic to PPD (152). The Leuven results could possibly have been explained by more frequent use of hair dye or temporary "black henna" tattoos than patients in other countries, including Sweden, but the use of hair dye was significantly more common in the Malmö patients than in those in Leuven ($P < 0.001$). Unfortunately, no information was found on the frequency of hair-dyeing and the kind of hair dyes used in the two countries.

Furthermore, more patients in Malmö than in Leuven had worked with finished textiles ($P < 0.001$), and these patients also had a contact allergy to PPD more often than patients who had not reported having had this occupation. On the other hand, more Belgian than Swedish patients reported that they had acquired temporary "black henna" tattoos although the difference was not statistically significant (Leuven vs. Malmö, $P = 0.18$).

Childhood eczema was found to be an independent, statistically highly significant risk factor for textile-related skin problems (Table 13). However, as previously reported (160), the validated question "Have you had childhood eczema?" may overestimate the prevalence of childhood eczema in adult population surveys, and this might also have been the case in this study. Having had childhood eczema may also reflect a history of general skin sensitivity. Significantly more of the Malmö patients reported having had eczema as a child than in Leuven, 25% vs. 18%, respectively ($P = 0.014$). The same results were seen in a multicentre questionnaire study from 2008, where almost twice as many Swedish children as Belgian children, 13-14 years old, had had flexural eczema during the past 12 months (161).

5.5 Gender differences in patch test results

When evaluating the answers to the questionnaire in Study II, the women had more often had eczema as a child, they had more often dyed their hair and had worked more often with textiles. Women also tended to suspect textiles as the cause of skin problems more often than men. Possible explanations of their higher frequency of

self-reported skin problems resulting from textiles could be that women are more prone to skin irritation, or perhaps are more observant of skin manifestations than the average man, and that women have a tendency to wear more tight-fitting clothes, leading to increased friction and sweating. Women also use more hair dyes, cosmetics, and other skin care products which may cause skin irritation and/or contact allergies.

In Studies I and III, as in several other studies, a higher frequency of contact allergy to disperse dyes was found in women than in men (93,122,124,125). The higher frequency of sensitization to disperse dyes in women may reflect their tendency to dye their hair more often than men. Both Studies I and III demonstrated a significantly higher prevalence of contact allergy to PPD in women than in men ($P = 0.026$ and $P = 0.041$, respectively), but the reverse for contact allergy to BRM ($P = 0.007$ and $P = 0.028$, respectively). This raises the question of whether women are sensitized to PPD primarily through hair dyes and secondly react to the TDM, and whether men are primarily sensitized to BRM and secondly to the TDM.

5.6 Chemical investigations

5.6.1 Concentration of purified dyes and impurities

The presence of impurities in disperse dyes has been known for many years. In 1978, Fregert and Trulson described TLC analyses of 19 textile dyes; 13 of which contained two or more substances (162). In a French study carried out in 1986, in which commercial textile dyes were analysed with TLC, DY 3, DO 1, and DO 3 appeared to be pure, whereas DB 35, DR 1, and DR 17 were impure (22). Dyes from various suppliers also differed, although their CI number was the same. In the same year, Hausen and Brandão reported that TLC analyses of DB 106 revealed that dyed material contained 7 other compounds, two of which were blue dyes (113). More than 10 years later, in a review article from 2000, Hatch and Maibach encouraged researchers to verify the identity and purity of the dyes used for patch testing (93).

The HPLC investigation of the disperse dye patch test preparations in Study IV demonstrated that most of the 107 preparations contained only 20-50% of the stated concentration. Over half (66%) of the preparations had passed the expiry date when analysed, but no correlation was found between the expiry date and declining concentration. The patch test preparations prepared at our department in Malmö showed the same low concentrations of the declared disperse dyes, although they contained measured material that constituted 1.0% of the petrolatum patch test preparation. The analyses demonstrated that most of the raw materials used at our department in Malmö contained about 40-75% contaminants and/or other substances. These results are in accordance with studies performed earlier at our department, demonstrating that the isocyanate patch test preparations used at various

dermatology departments showed considerable variations in the content of isocyanates (163,164).

The impurities seen under UV radiation gave spots on the TLC plates, but it is possible that they only constituted a minor part of the considerable contamination visualized as multiple peaks when analysed with HPLC. Additional impurities, not seen in visible or UV light, giving rise to contact allergy may have been present. Uter at al. (118) reported that one supplier of disperse dyes stated that naphthalene sulphonate was used in DB 106 and DB 124. According to online information given by an Indian manufacturer of textile dyes, commercial disperse dyes can contain as much as 60% lignin sulphonate/lignosulphonate, another emulsifier. Analyses of the disperse dyes used in the current studies confirmed that lignin sulphonate was present in DB 106 and DB 124. However, this probably has little influence on the allergenic potency of these dyes, as lignosulphonate consists of large polar molecules (165) with low allergenic potential. Lignin sulphonate has been used for patch testing at our department on more than 400 patients with no positive reaction. Only one report was found in the literature on contact allergy to calcium lignosulphonate (166).

As shown by the TLC patch testing in Study V, impurities in dyes may give rise to positive reactions indicating an allergenic potential, at least regarding the impurities in DB 106 and DB 124. The various components in the patch test preparations can be isolated and identified based on TLC testing. The area of silica on a reference chromatogram containing the substance(s) causing the positive reaction can be scraped off, extracted and chemically analysed (139). It is important to use an eluent giving optimal separation of the ingredients into different spots on the chromatogram when utilizing thin-layer chromatograms for patch testing of various products (139). The nature of and similarities between the pink spots on the DB 106 and DB 124 chromatograms are not understood, as the sensitizers in the chromatograms have not yet been identified. Comparable investigations and patch testing with thin-layer chromatograms of other commercial disperse dyes would also be of interest to determine whether similar impurities with allergenic potential can be found in these disperse dyes.

5.6.2 Incorrect labelling

Some of the DO 3 patch test preparations in Study IV did not contain DO 3. TLC and HPLC analyses showed that these patch test preparations instead contained another orange dye. The TLC analysis clearly shows the difference between the 2 dyes (Figure 18 C). Le Coz et al. reported in 2004 at the European Society of Contact Dermatitis meeting in Copenhagen that test preparations labelled DO 3 contained DO 31 instead, when analysed with TLC and HPLC (167). When investigated with MS at our department, the 4 patch test preparations with divergent properties in

Study IV showed the same results. Consequently, DO 31, 0.5%, was used in the TDM and in the ingredient testing in Study I and Studies II/III, whereas the authentic DO 3, was used the for testing at the higher concentration of 1.0%. This probably influenced the results of the present studies, as the patch test results in Study III indicate that DO 3 is a better substance to use in the TDM than DO 31 ("DO 3").

The results of these studies raise the question of whether the patch test preparations should be changed so as to contain only the chemically defined disperse dyes without impurities. However, the disperse dyes used in the textile industry are impure (93), and the results presented here emphasize the importance of identifying and estimating the allergenic capacity of the various sensitizers responsible for allergic reactions from clothes and other textiles. This has also been stressed in other articles (93,118), in which the use of TLC is mentioned as an important tool for detecting the sensitizers in products. Patch testing with impure patch test preparations, with preparations containing concentrations other than that stated on the label, or with the wrong substance may have implications for the individual patient leading to difficulties in diagnosis and treatment. It may also result in misleading or incorrect advice with regard to general preventive measures concerning contact allergy and ACD arising from disperse dyes.

6. SUMMARY AND CONCLUDING REMARKS

The main aim of the work presented in this thesis was to investigate clinical and chemical aspects of contact allergy to disperse dyes. The most important findings in the thesis are as follows.

- The frequency of contact allergy to a textile dye mix consisting of 8 disperse dyes, Disperse Blue 35, Disperse Blue 106, and Disperse Blue 124, Disperse Yellow 3, Disperse Orange 1 and Disperse Orange 3, Disperse Red 1 and Disperse Red 17, in southern Sweden was 1.5%, which is comparable to that found in other studies on contact allergy to disperse dyes conducted in southern Europe.

- The high prevalence of allergic reactions to Disperse Orange 1 found in Study I was unexpected, whereas the prevalence of contact allergy to Disperse Blue 106 and Disperse Blue 124 was lower than expected based on the results of other studies.

- The textile dye mix used was as good a detector of contact allergy to the 8 disperse dyes as simultaneous patch testing with the individual dyes at the concentrations used in the mix.

- Self-reported textile-related skin problems showed a non-significant tendency towards an association with contact allergy to the textile dye mix used, whereas contact allergy to p-phenylenediamine was shown to be a statistically significant risk factor for such skin problems.

- Other risk factors for patient-reported textile dermatitis were female gender, increasing age, and previous childhood eczema.

- Chemical investigations of the disperse dye patch test preparations from 13 dermatology departments around the world revealed that they contained varying and lower concentrations of the dyes than the concentration stated on the label, and that all preparations contained impurities.

- Disperse Orange 3 could not be demonstrated in some preparations labelled Disperse Orange 3, and the chemical analyses revealed that the Disperse Orange 3 used for testing at the concentration used in the mix at four departments was actually Disperse Orange 31.

- Patch testing with dilution series of purified Disperse Blue 106 and Disperse Blue 124 identified them as strong allergens.

- Patch testing with thin-layer chromatograms demonstrated that about a quarter of the patients diagnosed as having a contact allergy to Disperse Blue 106 and Disperse Blue 124 in fact reacted to impurities in the patch test preparations and not to the purified dyes.

On the basis of these findings the following remarks can be made.

When a patient shows a positive reaction to p-phenylenediamine or black rubber mix in the baseline series, the patient's history must be scrutinized to determine the possibility of textile-related skin problems, and testing with textile dyes should be considered.

A study using a textile dye mix composed of the same 8 disperse dyes but at higher concentrations than in the mix described here, and including the authentic Disperse Orange 3 has recently been concluded in Malmö and Leuven. The results of this study will hopefully provide sufficient information for deciding whether a modified mixture of textile dyes should be included in the baseline series.

According to information from some European clothing manufacturers (personal communication), the textile dyeing industry has been forced to stop using disperse dyes with known allergenic properties and to change to other dyes not listed as contact allergens. However, there is a risk that allergenic disperse dyes are still being used to dye textiles and other products in non-European countries. Furthermore, there is a need to investigate possible impurities in disperse dyes in order to define the capacity of the different components in the dyes to cause allergic reactions. Testing with thin-layer chromatograms can provide important information on whether a patient is allergic to the declared substance or to impurities. It is also important to determine whether the substance to which the patient reacts is used in non-textile products in order to be able to give patients correct advice related to their daily life.

Finally, I agree with Malin Frick-Engfeldt, who stated at the end of her thesis that, "despite 100 years of patch testing, the following questions remain: Do we really know with what we are testing and do we always know how to detect positive reactions?" (168). I would like to add the question: Do we always know the true allergen(s) in the patch test preparations used?

SUMMARY IN SWEDISH

Kliniska och kemiska studier av kontaktallergi mot dispersionsfärgämnen

Färg har alltid varit viktig för människan. Fägade textilier har återfunnits i 5000 år gamla gravar i Egypten. De färger som då användes kom från djur, växter eller mineraler. Det första syntetiska färgämnet upptäcktes i England av William Perkin 1856. Redan 1868 rapporterades om hudreaktioner där textilfärgämnen misstänktes som bakomliggande orsak. På 1940-talet rapporterades om ”epidemier” av kontaktallergi för det svarta färgämnet p-fenylendiamin (PPD) i strumpor, på 80- och 90-talet mot mörka kläder. Från början rörde det sig om fallrapporter, men de senaste decennierna har större patientmaterial studerats, speciellt i Sydeuropa. Motsvarande studier av förekomsten av kontaktallergi mot textilfärgämnen i norra Europa har saknats. Vid en kontaktallergi kan patienten få eksem eller enbart klåda utan synliga förändringar på exponerad hud. Kontaktallergin kan missas om patienter med hudbesvär inte rutinmässigt allergitestas med så kallat lapptest (epikutantest) för textilfärger.

De textilfärgämnen som rapporterats orsaka mest eksem är dispersionsfärgämnen. Dessa färgämnen används för att färga syntetfibrer. Man har vid rutinmässig lapptestning av patienter med hudbesvär sett positiva reaktioner hos 1-5%. Enligt the European Society of Contact Dermatitis bör man överväga att inkludera ett ämne i den så kallade basserien, den lapptestserie som patienter rutinmässigt testas med vid misstanke på kontaktallergi, om 0,5-1 % av patienterna uppvisar en allergisk reaktion vid lapptestning med det aktuella ämnet. Innan en sådan rekommendation kan övervägas för dispersionsfärgämnen behövs ytterligare studier.

Det övergripande syftet med avhandlingen har varit att bidra med kunskap om diagnostik och praktisk betydelse av kontaktallergi mot dispersionsfärgämnen. Avhandlingen grundar sig på 5 vetenskapliga uppsatser.

Alla patienter som utreddes vid Yrkes- och Miljödermatologiska avdelningen, Universitetssjukhuset MAS, Malmö (YMDA) 1999-2003 testades även med en textilfärgmix (TDM) bestående av 8 dispersionsfärger, Disperse Blue (DB) 35, 106 och 124, Disperse Yellow (DY) 3, Disperse Orange (DO) 1 och 3, Disperse Red (DR) 1 och 17 (delarbete I). 1,5% av de 3325 patienter som testades reagerade mot mixen. DO 1 var det vanligaste allergiframkallande färgämnet. Det finns få studier där detta färgämne tidigare har testats. Färre patienter än förväntat hade en allergisk reaktion mot DB 106 och DB 124, trots att dessa färgämnen i flera andra studier har ansetts vara viktiga kontaktallergen. En orsak till att så få patienter med kontaktallergi

för DB 106 och DB 124 fångades kan vara att de testades med för låg koncentration i vår studie.

Frånsett den första studien har forskningsprojektet skett i samarbete med allergienheten vid hudkliniken, Universitetssjukhuset, Leuven, Belgien. Under 2005 fick 475 patienter som lapptestades vid kliniken i Malmö och 383 patienter vid hudkliniken i Leuven besvara en enkät innan lapptestning (delarbete II). Syftet var att undersöka om det fanns ett samband mellan patienternas upplevda hudbesvär och kontaktallergi mot dispersionsfärgämnen, PPD eller vissa gummikemikalier som är kemiskt besläktade med PPD och som ingår i black rubber mix (BRM). 18% av patienterna misstänkte att kläder orsakade deras hudbesvär. Ett signifikant samband fanns mellan upplevda hudbesvär och kontaktallergi mot PPD. En tendens fanns även för ett samband mellan hudbesvär och kontaktallergi för mixen med textilfärgämnen men detta samband var inte signifikant. Kontaktallergi för BRM var för ovanligt för att utvärderas. Kvinnligt kön, stigande ålder och om patienten hade haft eksem som barn var enskilda faktorer som var kopplade till en högre förekomst av upplevda hudbesvär av kläder. Patienterna uppgav också att klädesplagg av syntet oftare gav hudproblem än kläder av andra material.

En av frågeställningarna i avhandlingen var om mixen av 8 dispersionsfärger kunde fånga patienterna med kontaktallergi för de enskilda färgämnena. Under 2004-2005 testades därför 1780 patienter som utreddes på misstanke om kontaktallergi vid klinikerna i Malmö och i Leuven, förutom med basserien också med TDM, med dess ingående komponenter separat och med motsvarande färgämnen i högre koncentration (delarbete III). 35 patienter (2,0%) var allergiska för mixen och 34 patienter reagerade för minst en ingrediens testad i samma koncentration som i mixen. TDM fångade minst lika många patienter som varje kombination av de 8 ingredienserna när de testades i samma koncentration som i mixen. När DB 106 och DB 124 testades i 10 gånger högre koncentration upptäcktes 5 respektive 4,5 gånger så många patienter med kontaktallergi.

För att kunna jämföra resultaten från lapptestning av patienter i olika studier är det viktigt att undersöka de testsubstanser som används vid olika centra. Flera studier har påvisat att dispersionsfärgämnen som används för testning inte är rena. För att undersöka detta närmare insamlades testpreparationer från kliniken i Malmö och från ytterligare 12 hudkliniker representerande 10 länder i olika delar av världen (delarbete IV). Samtliga 107 testpreparationer analyserades med högtrycksvätskekromatografi och tunnskiktskromatografi (TLC) på laboratoriet vid YMDA och jämfördes med referenssubstanser som upprenades och identifierades vid klinikens laboratorium. Medelkoncentrationen av referenssubstans i sprutorna som var märkta 1,0 eller 1% var lägre än förväntat och varierade från 0,25% för DB 124 till 0,68% för DO 3. Koncentrationen av referenssubstans varierade också mellan sprutor som enligt

märkningen innehöll samma färgämne. I samtliga testpreparationer påvisades föroreningar. I 4/15 testpreparationer märkta DO 3 kunde inte detta färgämne påvisas. Senare analyser med masspektrometri vid YMDA har visat att dessa sprutor innehöll DO 31 i stället.

Då kontaktallergi för DB 106 och DB 124 relativt ofta ger hudbesvär valde vi att studera dessa färgämnen närmare. Patienter med kontaktallergi för DB 106 och/eller DB 124 testades med kommersiellt och upprenat färgämne i spädningsserie samt med kommersiellt färgämne som hade separerats med hjälp av TLC på en speciell plastfilm belagd med kiselgel (delarbete V). Remsorna sattes mot huden som lapptest. Vid avläsning av testerna såg man vilken del av färgämnet som patienterna reagerade på. Under 2006 testades totalt 21 patienter, 11 patienter i Malmö och 10 i Leuven. Resultaten från testningen bekräftade tidigare studier som har visat att DB 106 och DB 124 är starka kontaktallergen. Cirka 25 % reagerade enbart för kommersiellt färgämne i spädningsserie och för andra fläckar än huvudfläcken på tunnskiktsplattorna. Resultaten innebär att dessa patienter inte är allergiska för det rena färgämnet utan för andra beståndsdelar i färgen.

Med utgångspunkt från de resultat som presenterats i avhandlingen kan vi konstatera att:

Förekomsten av kontaktallergi för dispersionsfärgämnen i södra Sverige kan jämföras med förekomsten i Sydeuropa.

Om en patient vid rutinmässig testning med basserien reagerar för PPD och/eller BRM, bör man tänka på att patientens hudbesvär kan vara relaterade till allergi för textilfärgämnen. Lapptestning med textilserien bör då övervägas.

Om endast en testlapp kan sättas för att påvisa kontaktallergi för de 8 dispersionsfärger som ingår i TDM är den mix som vi använt i våra studier tillräcklig som screening. För att ge oss ett beslutsunderlag om en mix ska införas i den rutinmässiga testningen av patienter med hudbesvär krävs dock ytterligare studier med en modifierad mix, där ingående komponenter inklusive den autentiska DO 3 testas i högre koncentration.

För att kunna bedöma resultaten från testning av patienter i olika studier är det viktigt att undersöka testsubstansernas innehåll. Våra undersökningar visade att medelkoncentrationen av referenssubstans i testsprutor med dispersionsfärgämnen som används vid olika hudmottagningar i världen var lägre än förväntat och varierade mellan olika mottagningar, att samtliga sprutor innehöll föroreningar och att vissa

sprutor innehöll fel substans. Detta ger en tankeställare när resultaten från olika studier jämförs.

Även om användandet av vissa allergiframkallande textilfärgämnen sägs ha minskat i de textilier som produceras för försäljning inom EU-området kan det finnas en risk att dispersionsfärgämnen med sådana egenskaper fortfarande används i tyger och kläder som säljs både i Europa och i andra världsdelar. Därför är det viktigt att undersöka om de färgämnen som används i textilier och i andra varor idag har allergiframkallande egenskaper. Testning med tunnskiktskromatogram kan ge information om patienten i så fall reagerar på den deklarerade substansen eller på någon förorening i färgen. Undersökningarna är viktiga för att kunna ge patienter med kontaktallergi mot dispersionsfärgämnen rätt information om vad de bör undvika i sitt dagliga liv.

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