Are allergenic disperse dyes used for dyeing textiles?

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doi:10.1111/j.1600-0536.2012.02129.x

Summary

Background. There are no data showing that disperse dyes, used to patch test patients, are currently being used for dyeing synthetic garments. It is unknown whether disperse dyes, which are currently routinely patch tested, are in fact present in synthetic textiles on the market.

Objectives. To determine whether eight disperse dyes, hitherto most widely cited as allergenic, are still used in textiles that are sold in various countries.

Methods. Textiles from 13 countries in Europe, Asia and the United States were analysed. The procedure used for dye identification was thin-layer chromatography. When there were matching spots from the textile extract and reference dye, high-performance liquid chromatography was performed.

Results. Of 121 analysed items, three showed positive results for some of the investigated disperse dyes. Four dyes in these items could be detected and confirmed by the use of high-performance liquid chromatography. A pair of light brown ladies' tights manufactured and purchased in Italy contained Disperse Yellow 3, Disperse Blue 124, and Disperse Blue 106, and a set of black bra and panties purchased in India contained Disperse Orange 1.

Conclusions. The eight disperse dyes that are most frequently incriminated in textile dye dermatitis are very rarely used in textiles nowadays.

Key words: allergic contact dermatitis; disperse dyes; textiles.

Disperse dyes (DDs), especially those belonging to the azo class, are the most prevalent cause of textile-related allergic contact dermatitis (1). Synthetic fabrics from fibres made entirely of or of polyester blended with polyester, acetate and nylon are dyed with DDs (2). Within the EU, azo dyes that can be metabolized to carcinogenic aromatic amines are forbidden, but up to now there is no legal prohibition on the use of allergenic azo dyes in any country (3). Approximately 50 dyes have been identified as contact allergens, and two-thirds of these are DDs, although they represent a very small fraction of the total of approximately 8000 commercially used dyes (4). According to the EU regulations for eco-labelling of textile products, the manufacturer of the garments produced in or imported to the EU must either provide a statement of non-use of certain DDs, listed as carcinogenic or allergenic, or provide a test report proving their colour fastness (5). The Oeko-Tex Association, a group of 14 textile institutes in Europe and Japan, has a list of allergenic dyes that are forbidden in clothes certified with the ecological Oeko-Tex label (6) (Table 1). In the United States, textile products that are produced in accordance with the Global Organic Textile Standard (GOTS) may be sold as organic. The GOTS prohibits the use of azo dyes

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Conflicts of interest: The authors have declared no conflicts. Funding: The study was supported by a scholarship to L. Malinauskiene from the Swedish Institute and by grants from the Swedish Asthma and Allergy Association's Research Foundation, the Edvard Welander Foundation and the Finsen Foundation.

Accepted for publication 15 May 2012

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Table 1. Disperse dyes classified as allergens and listed by the EU Commission and by Oeko-Tex

C.I. generic name	C.I. structure name	CAS number
C.I. Disperse Blue 1	C.I. 64 500	2475-45-8
C.I. Disperse Blue 3*	C.I. 61 505	2475-46-9
C.I. Disperse Blue 7	C.I. 62 500	3179-90-6
C.I. Disperse Blue 26	C.I. 63 305	–
C.I. Disperse Blue 35*†	–	12222-75-2
C.I. Disperse Blue 102	–	12222-97-8
C.I. Disperse Blue 106*†	–	12223-01-7
C.I. Disperse Blue 124*†	–	61951-51-7
C.I. Disperse Brown 1*	–	23355-64-8
C.I. Disperse Orange 1†*	C.I. 11 080	2581-69-3
C.I. Disperse Orange 3*†	C.I. 11 005	730-40-5
C.I. Disperse Orange 37	C.I. 11 132	–
C.I. Disperse Orange 76	C.I. 11 132	–
C.I. Disperse Red 1*†	C.I. 11 110	2872-52-8
C.I. Disperse Red 11	C.I. 62 015	2872-48-2
C.I. Disperse Red 17*†	C.I. 11 210	3179-89-3
C.I. Disperse Yellow 1	C.I. 10 345	119-15-3
C.I. Disperse Yellow 3*†	C.I. 11 855	2832-40-8
C.I. Disperse Yellow 9*	C.I. 10 375	6373-73-5
C.I. Disperse Yellow 39	–	–
C.I. Disperse Yellow 49	–	–

CAS, Chemical Abstract Service number, C.I., Colour Index number; – , no C.I. or CAS number for that dye.
*Present in the Textile Colours & Finish (TF-1000) series of Chemotechnique Diagnostics, Sweden.
†Investigated in the study.

that release carcinogenic arylamine compounds and DDs classified as allergenic (7, 8). In the EU, an average of 41% of clothing is imported from other countries with lower labour costs (9). There is a discrepancy between the member states in clothing import: in Sweden and Denmark, 90% of clothing products come from outside the EU, but in Portugal, almost all clothing products purchased are made in the EU (10). For the textile to be labelled 'Made in . . .' according to the EU legislation, two significant processes of manufacturing (i.e. textile spinning, weaving, finishing, or sewing) should be performed in that particular country (9).'Made in' labels are currently voluntary in the EU, and their use depends on national laws. In comparison, country of origin labelling is strictly regulated in, for example, the United States, Canada, and Japan. Nevertheless, the products imported into the EU should comply with the EU legislation. The main supplier of clothing to the EU is China, followed by Bangladesh, Pakistan, and Turkey (9). EU regulations are only applied in the EU countries, and do not have to be observed in countries outside the EU, where travellers can be exposed to garments dyed with these DDs.

There are sparse data on the presence of the dyes to which individuals are patch test-positive in the garments suspected as being the cause of their dermatitis. Chemical investigation of the textiles from the textile dye patch test-positive patients showed that dyes to which patients were patch test-positive were infrequently identified in their clothes (11). In spite of speculations that the allergenic DDs are probably no longer used, there are many reports of positive reactions to these DDs in the literature, and clinical relevance in most of the studies is considered to be high (11–14).

The primary objective of this study was to determine whether the DDs most frequently cited as allergens are used for dyeing textiles, especially in low-price garments.

Materials and Methods

Textiles

Dermatologists in different countries were contacted and asked to send us cheap socks, T-shirts, underwear, scarves, tights, etc., made from 100% polyester or blend with other synthetic fibres (e.g. polyamide), preferably with yellow, orange, or dark colours.

A total of 121 garments, mainly scarves, caps, T-shirts, socks, tights, trousers, jackets, skirts, dresses, and panties, were obtained from 13 countries. Their main characteristics are described in Table 2.

Chemicals

Chloroform and acetone of analytical grade were obtained from Scharlau Chemie S.A. (La Jota, Barcelona, Spain). Acetonitrile of fluorescence high-performance liquid chromatography (HPLC) grade was obtained from Lab-Scan (Dublin, Ireland). Distilled water was obtained from Millipore SA (Malsheim, France). Eight commercial DDs (Disperse Red 1, Disperse Red 17, Disperse Blue 106, Disperse Blue 35, Disperse Blue 124, Disperse Yellow 3, Disperse Orange 1, and Disperse Orange 3) were bought from Chemotechnique Diagnostics (Vellinge, Sweden). The reference substances for each of the DDs were isolated and identified at the Department of Occupational and Environmental Dermatology, Lund University, Skåne University Hospital, Malmö (15). The purity of the reference substances was >99%, except for Disperse Orange 3, which had a purity of >97%. We were not able to isolate and identify any reference substance for Disperse Blue 35, which is a mixture of different substances and belongs to the anthraquinone class of DDs. For comparison, we selected eight DDs that have been used for patch testing dermatitis patients in our department since 1999. Portuguese dermatologists proposed that these dyes should be included in the textile

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Table 2. The main characteristics of the analysed garments

Country of purchase, number of garments	Country of origin, number of garments	Colour, number of garments	Fibre composition, number of garments
Sweden, 26	China, 29	Black, 35	100% polyester, 32
Spain, 17	Italy, 12	Orange, 24	Polyester blends*, 26
Italy, 15	Cambodia, 5	Red, 19	100% polyamide, 4
Lithuania, 5	Argentina, 5	Brown, 9	Polyamide blends†, 12
Portugal, 4	India, 5	Blue, 8	100% acrylic, 1
Bulgaria, 1	Portugal, 3	Green, 6	Elastane blends‡, 5
Argentina, 7	South Korea, 3	Yellow, 5	No identification of the textile fibres in the labels, 41
Singapore, 6	Turkey, 2	Violet, 3	
China, 10	Spain, 2	Grey, 2	
South Korea, 4	Bangladesh, 2	Multicoloured, 10	
India, 14	Hungary, 1		
South Africa, 6	Philippines, 1		
Canada, 6	No information in the labels, 51		

*Polyester blends with elastane (synonym lycra), acrylic, polyamide (synonym nylon), cotton, and viscose.
†Polyamide blends with elastane or acrylate fibres.
‡Elastane blends with cotton and viscose.

dye mix, on the basis of reports in the literature and their own experience (F Brandão, Portugal, 2011 personal communication).

All solutions and thin-layer chromatography (TLC) plates were prepared from the same batches at our department.

Extracts from the textiles

The extracts were made from 20 × 20 cm of textile, cut into 1–2 cm pieces. These were put into a glass jar with a diameter of 6 cm, to which 150 ml of 100% acetone was added. Extraction was performed with an ultrasonic bath for 15 min. The extract was then vacuum-evaporated in a rotary evaporator until dry (30°C). The obtained residues were diluted in 1–2 ml of acetone and used for application to TLC plates.

TLC of sample extracts

One millilitre of extract was repeatedly applied on the silica gel on a 20 × 20 cm glass plate (60 F 254; thickness of the TLC layer, 250 µm; Merck KgaA, Darmstadt, Germany) with a micropipette, until 5–10 µl had been deposited for each spot every 2.5 cm along a line on the lower part of the silica gel plate.

A mixture of chloroform and acetonitrile, 86:14 (vol/vol), or 100% chloroform was used as the mobile phase. The separated components in the extracts gave a band of well-defined and well-separated spots. The TLC plates were all inspected under visible and ultraviolet (UV) light (254 and 366 nm, respectively).

Purified and commercial solutions of Disperse Red 1, Disperse Red 17, Disperse Blue 106, Disperse Blue 124, Disperse Yellow 3, Disperse Orange 1, Disperse Orange 3, and Disperse Blue 35 (only commercial), 1% in acetone, were used as reference substances.

If there were matching spots from the textile extract and the reference (purified) dye, TLC was performed, comparing extract, reference dye, and the mixture of the equal parts of the extract and the reference dye. If the matching of the spots on the TLC plate remained, HPLC of the extract and the reference dye was performed.

High-performance liquid chromatography

A ThermoFinnigan system was used, consisting of a P4000 quaternary pump, a UV 6000 diode array detector, an AS3000 autoinjector, an SN4000F control module, and software controlled by ChromQuest 4.1 and monitored by Spectral Analysis for ChromQuest (ThermoFinnigan, San José, CA, USA). The injection volume was 20 µl. The column (4.6 mm internal diameter × 250 mm) was packed with Kromasil (100 Å, 5 µm; Eka Nobel, Bohus,

Table 3. The linear gradient elutions used for high-performance liquid chromatography analysis of the disperse dyes*

Time (min)	%A	%B	Flow rate (ml/min)
0	60	40	1.0
35	0	100	2.0
45	0	100	2.0

*Solvents A and B consisted of acetonitrile/water 40:60 (vol/vol) and acetonitrile, respectively.

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Sweden). The detector scanned the eluate in the range 200–800 nm. HPLC was performed with a linear gradient of mobile phases, as described in Table 3. The identity of the substance producing a certain peak was determined by retention time and UV spectrum. The detection limit was 0.0001 μg/cm^2.

Results

Disperse dyes indicated by the TLC analysis

In total, 121 extracts were analysed by TLC. Three extracts were almost colourless and gave no visible spots on TLC plates.

A total of 31 extracts had similar spots to the main spots of the reference dyes on TLC plates. All spots on TLC plates were visible in daylight, and no additional spots were seen under UV light.

The majority of the 30 extracts had matching spots with Disperse Blue 124 (12 extracts), 10 with Disperse Orange 1, eight with Disperse Red 1, seven with Disperse Red 17, five with Disperse Orange 3 and Disperse Blue 106, and four with Disperse Blue 35 and Disperse Yellow 3. However, some of the extracts had matching spots not with the reference dye, but with impurities or with other substances present in the commercial dye preparation (Fig. 1).

Extracts that had similar spots to the main spot on the TLC of the purified reference dye were also analysed in another system. In one case, the results differed and there were no matching spots (Fig. 2).

Thirty extracts were mixed with the purified dye to confirm the presence of the reference dye. Mixes were 'positive' (that is, there was matching of the suspected dye spot from the extract and the reference dye on the TLC of the mix) in nine cases: in two with Disperse Yellow 3, Disperse Red 1, and Disperse Orange 1, and in one case each with Disperse Blue 124, Disperse Blue 106, and Disperse Orange 3 (Fig. 3).

Similar colour patterns

There was a similar pattern of dyes on TLC plates among different extracts, especially in the orange, blue, red and yellow spectra. TLC of the extracts made from different garments dyed in the same colour showed that the same colour could be composed of different dyes.

High-performance liquid chromatography

Ten samples were analysed by HPLC. It was not possible to confirm the presence of the suspected dye from the TLC analysis (Disperse Yellow 3, Disperse Orange 3, Disperse Blue 106, and Disperse Red 1) when evaluating the retention times and UV spectra in seven samples (Fig. 4). However, in brown tights, made and bought in Italy (made from polyamide and elastane), Disperse Yellow 3, Disperse Blue 106 and Disperse Blue 124 were confirmed by HPLC analysis. In a set of black bra and panties bought in India (material composition and country of manufacture were not present on the label), Disperse Orange 1 was detected (Fig. 5).

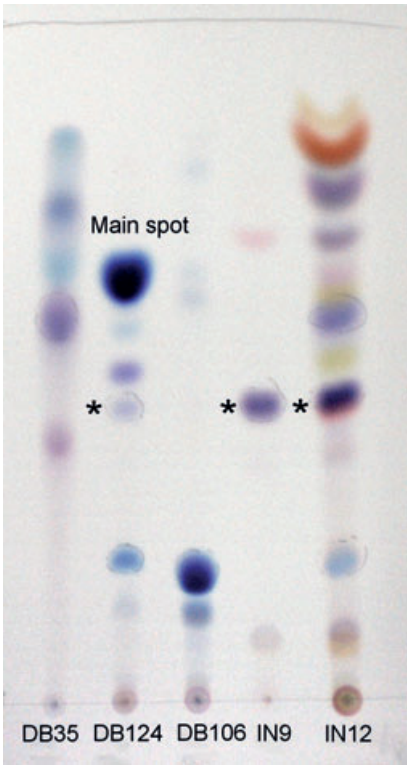

Fig. 1. Similar spots when thin-layer chromatograms of the extracts (IN9, IN12) and commercial Disperse Blue 124 (DB124) and Disperse Blue 106 (DB106) dyes are compared. These spots do not represent the main spot of DB124 or DB106. Asterisks (*) indicate similar colours.

Discussion

The prevalence of DD contact allergy varies with the population and the dyes tested. As DDs are used also in other applications, for example in toys, one study investigated commercially available toy samples (including textiles) from the market by liquid chromatography and tandem mass spectrometry for the presence of the allergenic and/or carcinogenic dyes listed by the EU, including Disperse Yellow 3, Disperse Blue 124, Disperse Orange 3, Disperse Blue 106, and Disperse Red 1.

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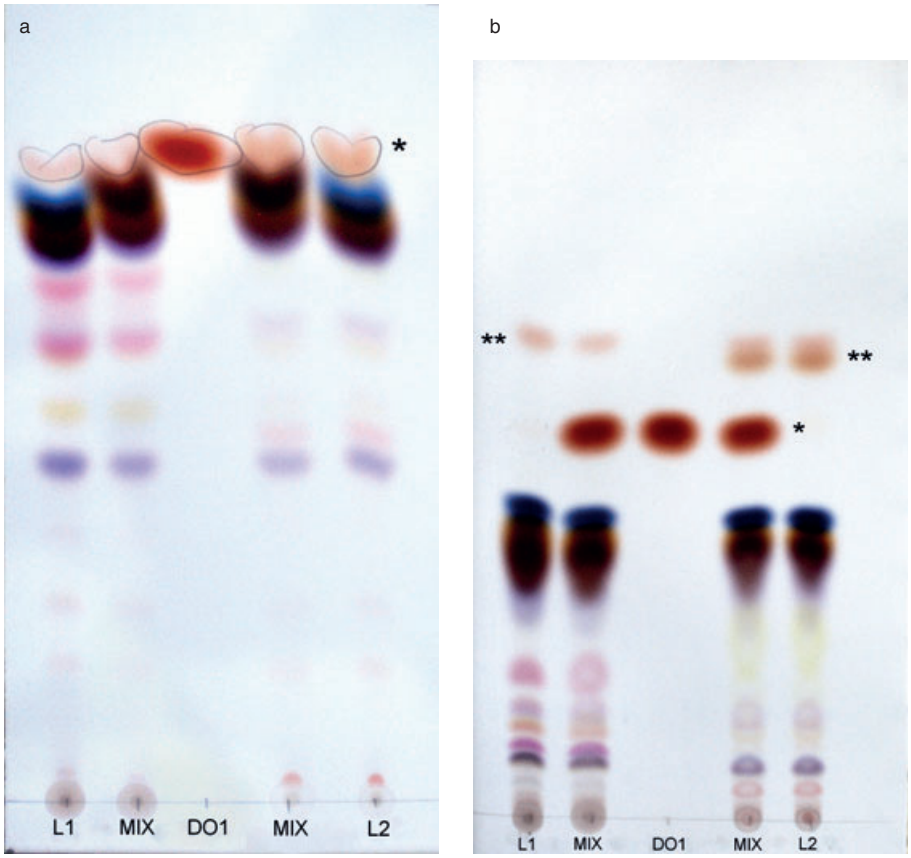

Fig. 2. Thin-layer chromatograms of the extracts (L1 and L2) as compared with purified Disperse Orange 1 (DO1) and mixtures (MIX) with it. Asterisks (*) indicate similar colours when the eluent system 84:16 chloroform/acetonitrile was used (a); when the system was changed to 100% chloroform (b), there were no matching colours.

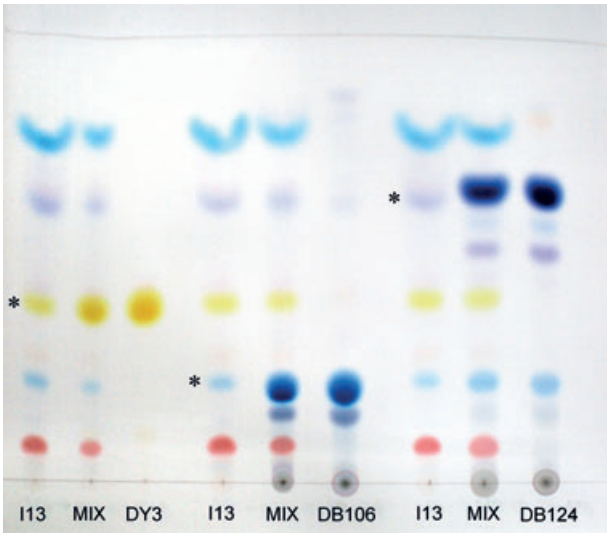

Fig. 3. Thin-layer chromatogram of the extract (I13) as compared with purified Disperse Yellow 3 (DY3), Disperse Blue 106 (DB106), Disperse Blue 124 (DB124), and mixtures (MIX) with them. Asterisks (*) indicate similar colours.

The authors were unable to find any of the investigated dyes (16). Therefore, it might be that allergenic DDs are also not used for dyeing textiles.

It was suspected that some of the eight DDs could be present in 30 of 121 (24.8%) extracts after initial TLC, but, using other methods (mixing the extract with the reference dye and repeating the procedure, changing the eluent system for TLC, and performing HPLC), we were able to confirm the presence of Disperse Yellow 3, Disperse Orange 1, Disperse Blue 106 and Disperse Blue 124 in three garments out of 121 (2.5%). As these items were obtained randomly, it is obvious that the DDs most commonly used for testing dermatitis patients are not widely used. Our study shows that one method for identification of the DD is not enough to confirm the identity of the dye. In one case, changing the eluent system for TLC from 86:14 chloroform/acetonitrile to 100% chloroform led to completely different results, and the spots did not correspond to those of the reference dye.

TLC showed that a textile is usually dyed with several different dyes, although it appears to have just one colour. The analysis also shows that the individual textile dyes usually contain impurities or that they are mixtures of

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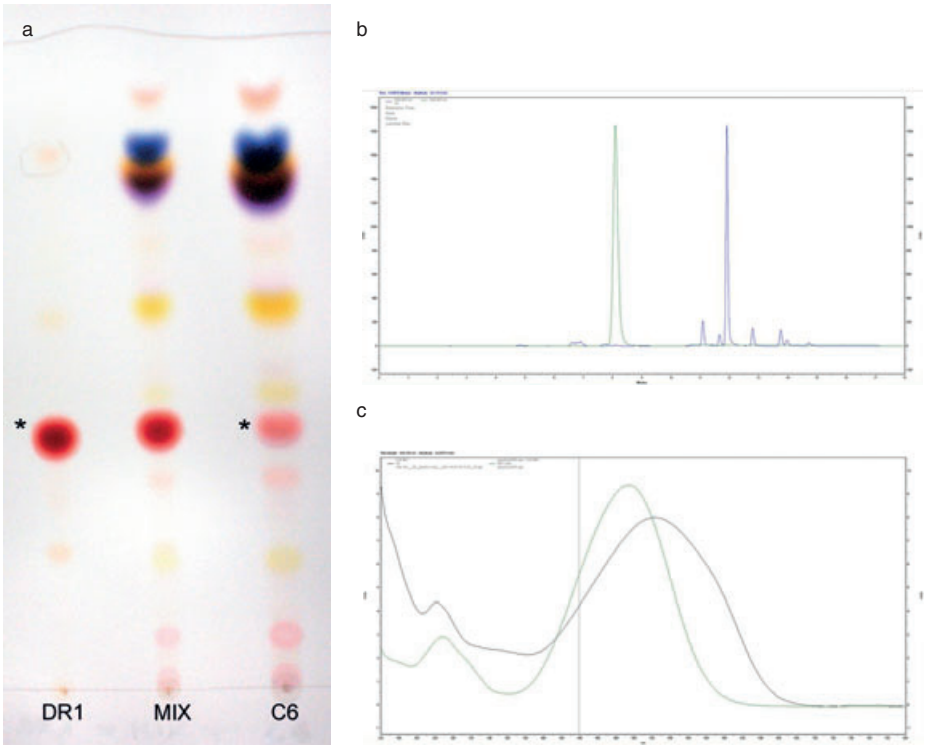

Fig. 4. (a) Thin-layer chromatogram of the extract from the textile (C6) as compared with the reference substance Disperse Red 1 (DR1) and a mixture of C6 and DR1 (MIX) indicates the presence of DR1 in C6, but this was not confirmed by high-performance liquid chromatography (HPLC). Asterisks (*) indicate similar colours. (b) HPLC chromatogram of purified DR1 (green line) and C6 (blue line) shows different retention times. (c) HPLC spectrogram recorded at a wavelength of 450 nm indicates that there is no DR1 (green line) in C6 (blue line); detection limit, ≥ 0.0001 μg/cm^2.

several dyes. The textiles that most often contain several different dyes are the black or dark ones. We found Disperse Orange 1 in the set of black bra and panties and Disperse Yellow 3, Disperse Blue 106 and Disperse Blue 124 in the brown tights. This shows that it can be difficult to predict which dyes are used in a textile just from its colour.

Thirty per cent of the garments, some of which were also obtained within the EU, had no fibre composition or country of origin information available on labels. Therefore, it may be difficult to avoid certain types of textile and thereby certain dyes for consumers.

When comparing the extracts with the commercial DDs on TLC plates, we found a significant number of the extracts with similar additional spots close to the main spot. These impurities, whether intentionally added substances or synthesis intermediates, might be relevant for the elicitation of allergic reactions. One study in Malmö showed that the raw material of DDs used for preparing patch tests contains 39–76% of contaminants or other substances (15). These can be relevant, as almost 25% of patients allergic to Disperse Blue 106 and Disperse Blue 124 did not react to the main spot but did react to the other spots on the TLCs made from the commercial forms of these dyes (17).

Our study was performed with randomly obtained items. One may suggest that investigating in a more systematic way (e.g. investigating only dark 100% polyester garments) would be more informative, but our goal was to obtain a general impression of the possibility of contact with certain disperse azo dyes in various garments.

For the investigation of textiles, we used two forms of chromatography: TLC and HPLC. TLC is simple to use, inexpensive, and quick. The TLC conditions can be easily modified to obtain efficient separation of different components in the mixture, but it is a qualitative and not a quantitative technique. It could be that overlapping of several components in the spot of the mixture with similar retention factors on the TLC plate occurs. This could be revealed by changing the TLC conditions (e.g. the eluent system) or by using other methods (e.g. HPLC). HPLC is most often a much more sensitive method – the detection limit of DDs in our study was 0.0001 μg/cm^2,

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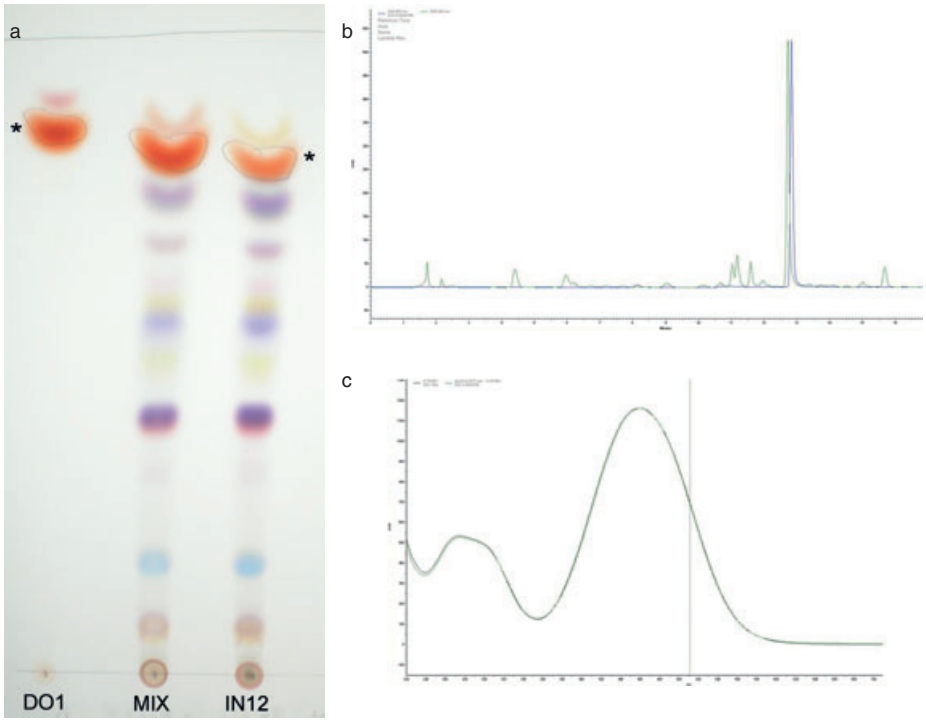

Fig. 5. (a) Thin-layer chromatogram of the extract from the textile (IN12) as compared with the reference substance Disperse Orange 1 (DO1) and a mixture of IN12 and DO1 (MIX) indicates the presence of DO1 in IN12. Asterisks (*) indicate similar colours.
(b) High-performance liquid chromatography (HPLC) chromatogram of purified DO1 (blue line) and IN12 (green line) shows very similar retention times. (c) HPLC spectrogram recorded at a wavelength of 450 nm indicates that DO1 (blue line) is present in IN12 (green line); detection limit, ≥ 0.0001 μg/cm^2.

and a standard textile garment contains approximately 100 μg/cm^2 of the dye (18).

It is very important to know whether dyes to which a patient has contact allergy are present in the textile. Indeed, in the majority of studies reporting positive reactions to DDs, clinical relevance is not stated, although it has been shown that dyes to which patients are infrequently patch test-positive are found in the suspected garment (11). Our study showed that some DDs usually implicated as allergens are infrequently found in the textiles. However, we were investigating the presence of the chemically defined dyes. Impurities that have been shown to be present in the commercial DDs, and thus in the patch test preparations, are also important in elicitation of the patch test reactions (17, 19, 20). DDs could constitute a marker of sensitization to other para-compounds [such as *p*-phenylenediamine (PPD) or black rubber substances].

For establishing the clinical relevance of positive patch test reactions to DDs, patch testing with either the suspected fabric or an extract from the fabric should be performed; that is, exposure to that DD should be confirmed. However, the extraction procedure is not standardized. In our clinic, we follow the procedure described in Materials and Methods. We use mainly acetone as a solvent for the preparation of the extracts from the textiles, because acetone can dissolve both polar and non-polar compounds, although any solvent suitable for patch testing may be used. The extraction procedure with an ultrasonic bath provides an improved technique with which to obtain more standardized extracts from the same kind of products (21).

Ideally, identification of the dye content in the suspected fabric should also be carried out, although this is not always possible. Indeed, the latest reports that we found when reviewing the literature on chemical analysis of the suspected textile were published in 2000 (22, 23).

On the other hand, the diagnosis of textile dye dermatitis can be missed even if patch testing with the commercial DD preparations is performed, because these DDs are uncommon in textiles. It has been reported that 18% of surveyed dermatitis patients suspect textiles (especially synthetic) as a cause of their skin problems, but there was no statistical correlation with positive patch test reactions to DDs (24). Interestingly, self-reported textile-related skin problems were statistically significantly

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associated with contact allergy to PPD, which is frequently found in cases with contact allergy to the azo dyes (24). Therefore, it could be that other disperse azo dyes are used in the textiles that we are wearing today.

Conclusions

In this study, the majority of the investigated fabrics were found not to contain any of the eight DDs present in our baseline series. However, it is still possible to find them, not only in the garments made outside the EU, but also in those made in the EU. Our study also shows that there is a need to determine which DDs are actually being currently used to dye textiles and that could be of clinical importance, and consequently should be included in patch test preparations used for detecting contact allergy to textile dyes.

Acknowledgements

The authors thank Alicia Cannavó (Argentina), Juan Chao (China), Caterina Foti (Italy), Ana Giménez-Arnau (Spain), Margarida Gonçalo (Portugal), Anthony Goon (Singapore), An Goossens (Belgium), Hemangi R. Jerajani (India), Dick Johnsson (Canada), H&M and Lindex for providing textiles. We also thank Lena Persson for her valuable technical assistance.

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