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INVESTIGATIVE REPORT

Patch Testing with a Textile Dye Mix in a Baseline Series in Two Countries

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Disperse dyes are the most common contact sensitizers among textile dyes. The main aim of this study was to investigate the outcome of patch testing with a textile dye mix 6.6%. A total of 2,049 patients from Sweden and 497 from Belgium were tested with the mix, consisting of Disperse (D) Blue 35, D Yellow 3, D Orange 1 and 3, D Red 1 and 17, 1.0% each, and D Blue 106 and D Blue 124, 0.3% each. Of the total number, 65 patients, 2.6%, tested positively to the mix, 4.2% of the Belgian patients and 2.1% of the Swedish patients. Patch testing with the mix 6.6% revealed significantly more patients with contact allergy compared with testing with a previous mix 3.2% ($p < 0.01$). Contact allergy to the mix was significantly more common in the Belgian than in the Swedish patients. **Key words:** Belgium; contact allergy; disperse dyes; *p*-phenylenediamine; Sweden, textile dye mix.

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Disperse dyes are used for colouring synthetic textile fibres. Although they are well-known contact sensitizers (1), they are not included in the majority of commercially available baseline patch test series (2). In two studies, one performed at the Department of Occupational and Environmental Dermatology in Malmö, Sweden, and one performed both at the Malmö department and the Department of Dermatology, Katholieke Universiteit, Leuven, Belgium, 1.5% and 2.0%, respectively, of consecutively patch-tested dermatitis patients were found to be allergic to a mix of textile dyes consisting of eight disperse dyes (3, 4). The mix used in the two studies was made of dyes that were labelled as containing Disperse (D) Blue 35, 106 and 124, D Yellow 3, D Orange 1 and 3, and D Red 1 and 17 (Fig. 1). However, chemical analysis, performed when the studies were terminated, revealed that the dye labelled D Orange 3 in reality was D Orange 31, whereas the other seven disperse dyes contained the dyes labelled (5, 6).

The rate of contact allergy found in the aforementioned studies raised the question of whether a textile dye mix (TDM) should be included in the baseline series (7).

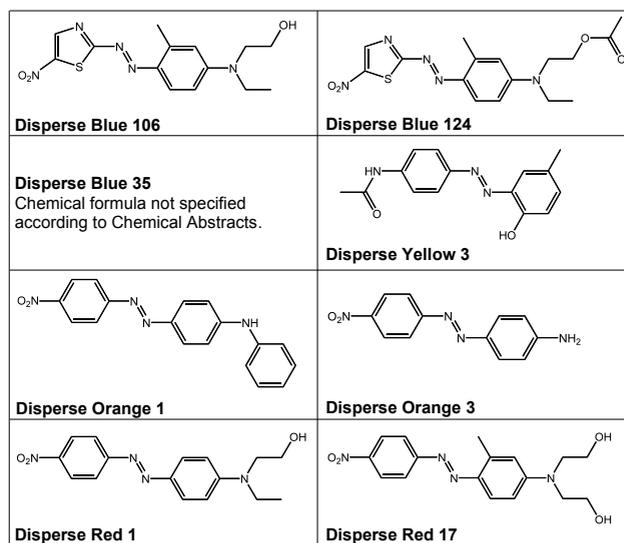


Fig. 1. Chemical structures of the eight disperse dyes in the textile dye mix.

However, to include the mix in the baseline series one must determine the optimal patch test concentrations of the ingredients in the mix.

The main aim of the present study was to investigate whether patch testing with a modified mix of textile dyes containing a higher concentration of the eight ingredients than previously used in the aforementioned studies would reveal more patients with contact allergy. A second aim was to compare the frequency of patients with allergic reactions to D Blue 106 and D Blue 124 when simultaneously patch-tested with these dyes at the concentration 0.3% w/w in petrolatum (pet.) used in the modified mix and at the higher concentration 1.0% w/w (pet.) used in commercially available textile patch test series. A third aim was also to compare the frequency of patients with contact allergy to the TDM at the departments in Malmö and in Leuven.

MATERIALS AND METHODS

Study population

The study population consisted of 2,546 consecutively patch tested dermatitis patients: 2,049 patients at the Department of Occupational and Environmental Dermatology, Malmö University Hospital, Malmö, Sweden from January 2006 until December 2008, and 497 patients at the Department of Dermatology, Katholieke Universiteit, Leuven, Belgium from January 2006 until August 2007. The demographic data on the patch-tested

patients, with 62.8% women (mean age 42.7, age range 12–94 years) and 37.2% men (mean age 43.4, age range 12–86 years), showed good agreement for the two centres.

Patch test preparations

The baseline series used at the Malmö department was purchased from Chemotechnique Diagnostics, Vellinge, Sweden. It included p-phenylenediamine (PPD) 1% w/w (pet.) and black rubber mix (BRM) 0.6% w/w (pet.), consisting of three components, N, N'-diphenyl-1, 4-phenylenediamine, N-cyclohexyl-N'-phenyl-1, 4-phenylenediamine, and N-isopropyl-N'-phenyl-1, 4-phenylenediamine, 0.2% w/w (pet.) each. The department in Leuven bought the baseline series, including PPD 1% w/w (pet.), from Trolab, Reinbek, Germany. BRM 0.6% w/w (pet.) used in Leuven was made by Chemotechnique Diagnostics, as this mix was not included in the baseline series from Trolab®. The TDM 6.6% w/w (pet.), consisting of D Blue 35, D Yellow 3, D Orange 1, D Orange 3, D Red 1, and D Red 17, 1.0% w/w (pet.) each, in addition to D Blue 106 and D Blue 124, each at 0.3% w/w (pet.), was temporarily included in the baseline series in both Malmö and Leuven. The eight dyes included in the mix were bought from Chemotechnique Diagnostics, and the mix and the separate dye preparations, which were used for the patch testing both in Leuven and in Malmö were prepared from the same batches at the Malmö department. The patch test preparations containing D Blue 106 and D Blue 124 1.0% w/w (pet.) each, which were used for patch testing in the study, were included in a textile colours and finish series purchased from Chemotechnique Diagnostics.

Patch test technique

Patch testing of the patients followed the routine of the two departments. For the patch testing with the TDM and the eight individual dyes Finn Chambers® (8 mm diameter; Epitest Ltd, Tuusula, Finland) on Scanpor® tape (Norgesplaster A/S, Venesla, Norway) were used both in Malmö and in Leuven (8). The test chambers were left on the patient's back for 2 days and readings were taken following the guidelines of the International Contact Dermatitis Group (9). Due to the different routines at the participating departments, readings were performed in Malmö on day 3 or 4 and on day 7 or 8, and in Leuven on day 2 and day 4. The patch test reactions on day 3 or day 4 were used for registration in the present study.

In Leuven, simultaneous patch testing was performed with the TDM, with its ingredients at the same concentration as in the mix and with D Blue 106 and D Blue 124 at 1.0% w/w (pet.). In Malmö, patients with positive reactions to the dye mix at the reading on day 3 or 4 were directly tested with the eight disperse dyes at the same concentration as in the mix and additionally with D Blue 106 and D Blue 124 1.0% w/w (pet.). The logistics of the study, including the numbers of patients who participated at the two departments, are shown in Fig. 2.

Statistical analysis

The results were analysed using SPSS version 16.0 (SPSS Inc. Chicago, IL, USA). Fisher's exact test or McNemar test was used, and we considered two-sided $p < 0.05$ to be statistically significant.

RESULTS

Consecutive patch testing with the textile dye mix in the baseline series in Leuven and Malmö

The reactivity to the TDM is shown in Table I. Contact allergy to the dye mix was found in 65 (2.6%) of the

2,546 patients, 2.1% of the Malmö patients and 4.2% of the patients from Leuven ($p < 0.01$) (Fig. 3). Fifteen patients had doubtful reactions to the TDM. No irritant reactions to the TDM or the separate disperse dyes were noted. Of the 65 mix-positive patients 62 were patch-tested with the ingredients in the mix. Forty-nine (79%) of these 62 patients were allergic to at least one ingredient in the mix at the patch test reading on day 3 or day 4. Seventy-one percent of the TDM-positive Leuven patients reacted to at least one ingredient, compared with 83% of the corresponding Malmö patients ($p = 0.15$). The most frequent disperse dye contact allergy in the mix-positive patients was D Orange 1, followed by D Orange 3 (Fig. 4). Three patients were allergic to D Blue 106 and 4 patients to D Blue 124 (0.3% w/w (pet.) each). Simultaneous contact allergy to D Blue 124 was found in all patients allergic to D Blue 106 (Table I).

Patch testing with the textile dye mix and the eight disperse dyes in the baseline series in Leuven

A total of 497 patients in Leuven were simultaneously patch-tested with the dye mix and the separate ingredients at the same concentrations as in the mix. Of these patients, 21 were allergic to the mix and 15 (71%) of these mix-positive patients were also allergic to at least one ingredient. In total, 19 patients reacted to at least one ingredient in the mix; thus the TDM missed 4 (21%) of the patients with contact allergy to at least one ingredient in the mix (Table I). In the four patients without any contact allergy to the dye mix, allergic reactions to D Blue 35 were registered in two patients, to D Orange 3 in one patient, and to D Blue 106 0.3% in one patient (Table I).

Patch testing with D Blue 106 and D Blue 124 at two concentrations in Leuven and Malmö

All 497 patients in Leuven and 40 of the 44 TDM-positive patients in Malmö were patch-tested both with D Blue 106 and D Blue 124 0.3% and 1.0% w/w (pet.)

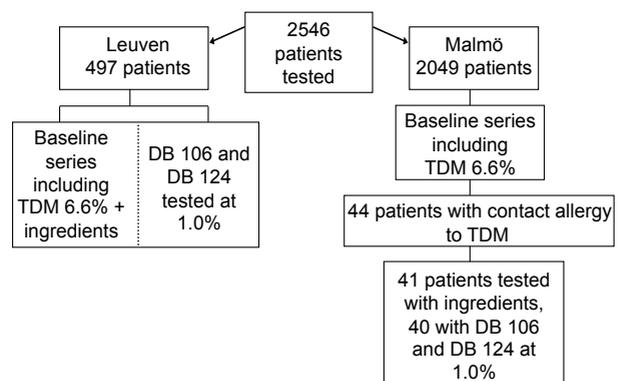


Fig. 2. Logistics of the study, including the number of patients who participated at the two departments. DB: Disperse Blue; TDM: textile dye mix.

Table I. Patch test results in: (i) the patients (numbers 1–44) from Malmö with positive reactions for the textile dye mix (TDM); (ii) the patients (numbers 45–71) with positive reactions for at least one of the following test preparations: the TDM, any of its eight ingredients tested at the concentration in the mix, or Disperse Blue (DB) 106 and/or DB 124 tested at 1.0% w/w (pet.); and (iii) the remaining patients (numbers 72–107) from Malmö and Leuven with positive reactions to *p*-phenylenediamine (PPD) 1% w/w (pet.). Test reactivity is denoted +, ++, or +++. Empty cells indicate negative test results

Pat. no.	TDM 6.6%	DB 106 0.3%	DB 124 0.3%	DB 35 1.0%	DY 3 1.0%	DO 1 1.0%	DO 3 1.0%	DR 1 1.0%	DR 17 1.0%	DB 106 1.0%	DB 124 1.0%	PPD 1%
1	+++				+++	+++	+++	+++	+++			+++
2	+++				++	+++	+++			NT	NT	+++
3	+++				+++	+++	+++	++				+++
4	+++				+	++	+++	+				+++
5	+++				+++	++	+++		++			+++
6	+++					+++						
7	+++	+++	+++		+++					+++	+++	
8	+++	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	+++
9	+++				+++							
10	+++						+++					
11–12	+++				++							
13	++						++					+++
14	++						+					+++
15	++				+	+	++					+++
16–20	++					++						
21–24	++					+						
25	++					++				++	+	
26	++	+	+	+						+		
27	++						+					
28	++	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	
29	++	++	++							++	++	
30	++				+		+					
31	++									+	+	
32–34	++											
35	+				+		++					+++
36–37	+					++						
38	+						+					
39	+				+							
40	+						++					
41	+	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	
42–44	+											
45	+++				++	+	+++					++
46	+++						+++					+++
47	+++						+++					+
48	++					+						+
49–50	++					++						
51	++		+							+		
52	++			+								
53–54	+						+					+ / ++
55	+				+	+	+	+				++
56	+					++						
57	+					+						
58	+					+						
59	+			+								
60–65	+											++(1)/(1)
66										+		+
67		+								+	+	
68				++								
69							+					+
70				+								
71										+		
72–73												+++
74–84												++
85–107												+

DO: Disperse Orange; DR: Disperse Red; DY: Disperse Yellow; NT: not tested.

each. A total of nine patients were allergic to D Blue 106 tested at the higher concentration, including all four patients reacting positively to D Blue 106 0.3%. The cor-

responding patch testing with D Blue 124 at the lower concentration, 0.3%, and at the higher concentration, 1.0%, revealed 4 and 5 patients with contact allergy,

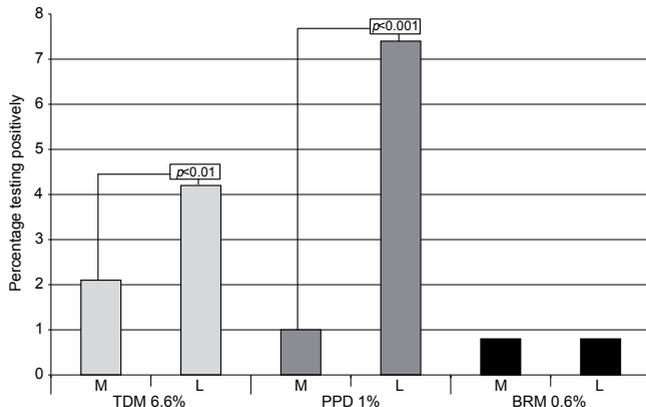


Fig. 3. Proportion (%) of contact allergy to the textile dye mix (TDM), p-phenylenediamine (PPD) and black rubber mix (BRM) in 2,546 patch-tested patients; 2,049 patients in Malmö (M) and 497 patients in Leuven (L), respectively.

respectively. However, only 2 of the 5 positive patients at 1.0% tested positive at 0.3% (Fig. 5).

DISCUSSION

Disperse dye mixes with various compositions have been used for patch testing in several studies in order to trace patients with contact allergy to textile dyes (3, 4, 10–13). A common conclusion of these studies has been that further investigations were necessary before deciding on whether a particular TDM should be added to the baseline series. The main aim of the present study was to compare the outcome of the patch testing with a TDM consisting of 8 disperse dyes when used in a higher concentration than the concentration used in two previous studies performed at our department (3, 4). In the present TDM 6.6% w/w (pet.), D Blue 35, D Yellow 3, D Orange 1, D Red 1, and D Red 17, were all tested at the double concentration compared with the corresponding ingredients in the TDM 3.2%, in addition to D Blue 106 and D Blue 124, both tested at 3 times the concentration compared with the concentration used in the previous dye mix.

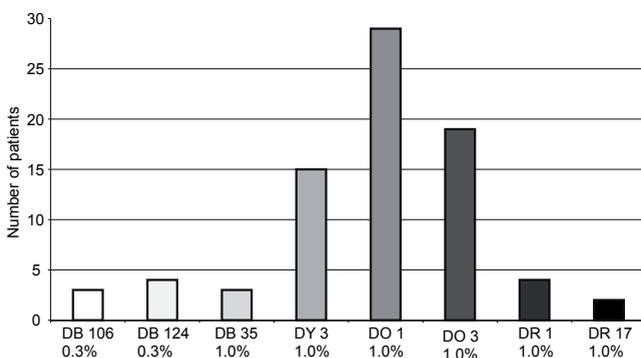


Fig. 4. Number of patients with positive patch tests to the eight disperse dyes in the textile dye mix (TDM) in 62 out of 65 patients testing positively to the mix. DB: Disperse Blue; DO: Disperse Orange; DR: Disperse Red; DY: Disperse Yellow.

Furthermore, the previous TDM 3.2% also contained an ingredient labelled D Orange 3 0.5% w/w (pet.). However, chemical investigations of the 8 disperse dyes performed at the Malmö department when the two aforementioned studies were finished revealed that the “D Orange 3” ingredient in the TDM 3.2% in reality was D Orange 31 (5, 6). No association was seen between contact allergy to D Orange 31 tested at 0.5% and to D Orange 3 simultaneously tested at the double concentration, 1.0%, in the previous study (4). The TDM 6.6% used in the present study contained D Orange 3 tested at 1.0% w/w (pet.). The consequences of patch testing with different ingredients in the previous and the present mix must be taken into account when evaluating and comparing the results of the different studies. However, the patch test results, with a low prevalence of contact allergy to D Orange 31 compared with a higher frequency of contact allergy to D Orange 3 in the previous study indicate that D Orange 3 is a better substance to use in the mix than D Orange 31 (4).

In the two previous studies, performed only in Malmö and both in Malmö and Leuven, respectively, 1.7% of 5,105 consecutively patch-tested patients were allergic to the TDM 3.2% (3, 4) compared with 2.6% to the TDM 6.6%. Accordingly, and as predicted, the TDM 6.6% revealed significantly more patients with contact allergy to the TDM ($p < 0.01$). Although the increase in frequency of patients with contact allergy to the TDM is probably due to the fact that the TDM was tested at a higher concentration in the present study, it may also be influenced by the fact that the TDM 6.6% contained D Orange 3 instead of D Orange 31. In fact, in the present study 8 out of 19 TDM-positive patients with contact allergy to D Orange 3 1.0% reacted only to this dye when tested with the ingredients in the mix.

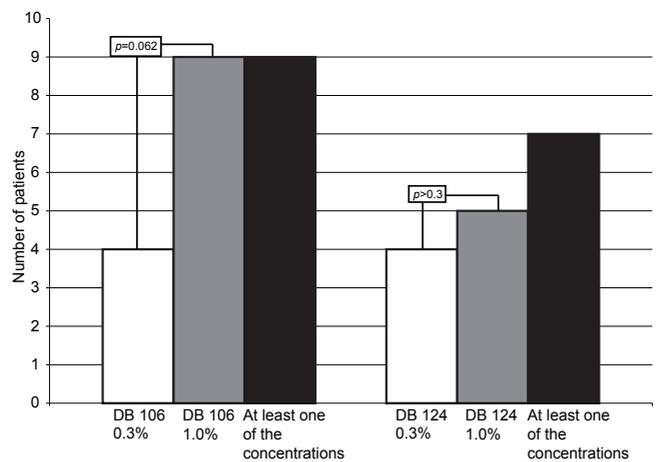


Fig. 5. Number of patients with positive patch test reactions to Disperse Blue (DB) 106 and DB 124 at the concentrations found in the mix, 0.3% w/w (pet.), to the corresponding dyes tested at 1.0% w/w (pet.), and to at least one of the two concentrations tested, in 537 patients. The p -values (McNemar) refer to comparisons between the number of patients with positive reactions for the two dyes at each of the two concentrations.

As also demonstrated in our previous studies, D Orange 1 was the most common allergen in the present study (Fig. 4) (3, 4). In the two previous studies 35% of the TDM-positive patients reacted positively to D Orange 1, compared with 47% in the present study. In this study D Orange 3 was the second most common allergen, while D Yellow 3 came second in the previous studies (4). In several other studies, however, D Blue 106 and D Blue 124 have been described as common allergens and many authors of studies on contact allergy to disperse dyes have recommended them as screening allergens for textile dye dermatitis (12, 14). One possible reason for the divergent results compared with the outcome of our studies may be that D Blue 106 and D Blue 124 were tested at a higher concentration, 1% (pet.) each (14) or in a mix of the dyes at 1.0% w/w (pet.) (12, 13, 15) in many of these studies. The outcome of our first studies, with a low frequency of contact allergy to D Blue 106 and D Blue 124 when tested in the mix at 0.1% (3, 4) was the reason for increasing the concentration to 0.3% in the TDM used in the present study. The simultaneous patch testing in the present study with D Blue 106 1.0% revealed 2.25 times as many allergic patients compared with testing with the lower concentration 0.3%. The corresponding patch testing with D Blue 124 1.0% revealed 1.25 times as many patients with contact allergy compared with patch testing with D Blue 124 0.3% w/w.

To identify patients with contact allergy to disperse dyes, but to avoid the risk of adverse reactions, particularly active sensitization, it is important to determine the optimal concentrations of the ingredients to be included in a modified dye mix (7, 16). Several circumstances must be considered, e.g. that the penetration into the skin of the ingredients, when present in the mix, can be higher compared with the penetration of the ingredients when tested separately. Compound allergy, synergistic

or additive effects between the substances have also been demonstrated when testing mixes e.g. fragrance-mix (17, 18). Furthermore, concerning D Blue 106 and D Blue 124, previous studies indicated that all patch test preparations of D Blue 124 contained D Blue 106 and vice versa (6, 19). In the present study, however, we are not aware of any case of active sensitization related to the disperse dyes tested in the present concentration.

To obtain a higher number of patients in the study, it was performed at two departments: in Malmö and Leuven. This also gave us the opportunity to compare the prevalence of patients with contact allergy to the TDM at the two departments. Contact allergy to the TDM was significantly more frequent in the patients from Belgium than in the Swedish patients ($p < 0.01$) (Fig. 3). However, the proportion of patients with contact allergy to the individual dyes in the mix in the TDM-positive Malmö patients was higher for all dyes, except for D Blue 35, compared with the proportion of patients with allergic reactions to the individual dyes in the corresponding Leuven patients (Fig. 6). Another explanation for a high frequency of allergic reactions to TDM could be simultaneous contact allergy to TDM and to other potentially cross-reacting allergens, such as PPD or BRM ingredients. The prevalence of allergic reactions to PPD was significantly more frequent in the Belgian than in the Swedish patients ($p < 0.001$). On the other hand, no statistically significant differences were seen between the number of patients in the two centres who reacted positively for BRM, which is a mix of three ingredients, all chemically closely related to PPD (Fig. 3).

In another study performed at the departments in Malmö and in Leuven where patients answered a questionnaire on past or present skin problems related to textiles and exposure to textile dyes and chemically related substances, contact allergy to PPD was also found to be a more important risk factor for textile-related skin problems than contact allergy to the TDM 3.2% (20). Consequently, it is uncertain whether the significantly higher frequency of contact allergy to the TDM 6.6% in the Leuven patients can be explained by the fact that the Belgian patients use other clothing and clothing materials containing more disperse dyes than the Swedish patients, or if it indicates a "cross-reactivity" between ingredient(s) in the TDM and PPD. At least some of the patients allergic to the TDM may initially have been sensitized to PPD and then reacting to disperse dyes due to cross-reactivity, or they may have been sensitized due to exposure to a common metabolite, rather than primarily sensitized to disperse dyes in textiles.

Ideally, the clinical relevance of a positive reaction to the TDM should have been noted in the reports from the participating departments. In the present study this registration was not done as this was not the purpose of the study. When assessing the clinical relevance, instruments such as the case history, communications

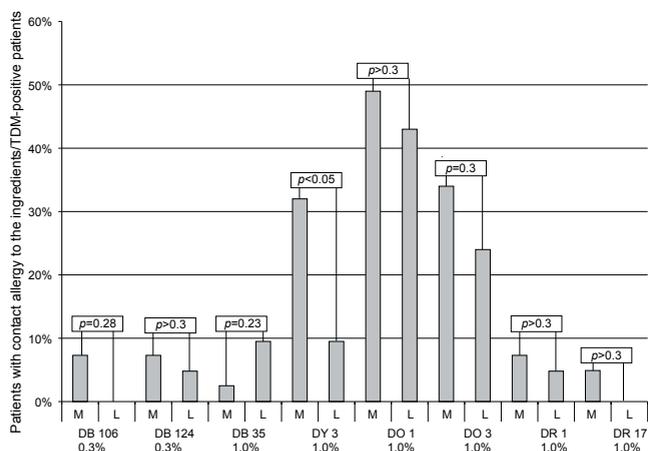


Fig. 6. Proportion (%) of contact allergy to the individual eight disperse dyes in the mix in those 41 patients in Malmö and 21 patients in Leuven with contact allergy to the textile dye mix (TDM). DB: Disperse Blue; DO: Disperse Orange; DR: Disperse Red; DY: Disperse Yellow.

with the manufacturers and chemical analyses of the suspected contactant should be given. It also requires the possibility to follow patients for an extended time to determine whether they clear when the incriminating agent is removed, e.g. textiles coloured with these disperse dyes. However, in most cases one or more of these steps are lacking. Furthermore, guidelines on the assessments of clinical relevance and tests for the same are still lacking (7). As we are aware of the problem of defining the clinical relevance of contact allergy to the disperse dyes used, we are planning a scientific study in which the patients will be exposed to textiles with a known content of these dyes.

Conclusion

The main purpose of patch testing with a mix is to simplify the patch testing and save space and time. As predicted, patch testing with TDM 6.6% in the present study detected significantly more patients with contact allergy compared with testing with TDM 3.2% ($p < 0.01$). All ingredients in the mix were tested at 1.0%, except for D Blue 106 and D Blue 124, which were tested at 0.3%. The reason for this was mainly a fear that these two dyes tested at a higher concentration should give very strong allergic reactions and partly a risk for active sensitization. However, we have not seen any indications of sensitization in this study. Patch testing with D Blue 106 and D Blue 124 at 1.0% would detect more patients with contact allergy. Therefore, patch testing should also be performed in consecutive dermatitis patients with a TDM with a 1.0% concentration of each ingredient, before a final decision is made about which textile dyes should be considered for inclusion in the baseline series.

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The authors declare no conflicts of interest.

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