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## INVESTIGATIVE REPORT

# Patch Testing with a Textile Dye Mix in Two Concentrations – A Multicentre Study by the Swedish Contact Dermatitis Research Group

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**Disperse dyes, which are used for colouring synthetic textile fibres, are well-known contact sensitizers. To investigate the outcome of patch-testing with a textile dye mix (TDM) at 7 dermatology clinics in Sweden, a TDM tested at 2 concentrations was included into the baseline series during one year. The mix consisted of Disperse (D) Blue 35, D Yellow 3, D Orange 1 and 3, D Red 1 and 17, all 1.0%, and D Blue 106 and D Blue 124, each 0.3% in the mix 6.6% and 1.0% each in the mix 8.0%. In 2,122 tested patients, contact allergy to the TDM at the concentration 8.0% was found in 2.8% and to the TDM at 6.6% in 2.5% of the patients. The contact allergy to the TDM could explain or contribute to the dermatitis in about 35% of the patients. Conclusion: contact allergy to the TDM is common and inclusion into the Swedish baseline series should be considered. Key words: allergic contact dermatitis; baseline series; clinical relevance; disperse dyes; p-phenylenediamine; simultaneous reactivity; textile dye mix.**

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Disperse dyes (DDs) are used for colouring synthetic textile fibres. Although these textile dyes are well-known contact sensitizers, they are not included in the majority of commercially available baseline patch test series. However, several DDs have been used for patch testing in various studies to detect patients with contact allergy to textile dyes (1–3). In a study from the United States published in 2012 the authors concluded that supplementing the baseline series with a textile series would increase the detection of patients with textile dye allergies (4). Mixes of DDs have also been used in several studies in order to identify patients with contact allergy (3, 5–10). The present multicentre study was initiated

by the Malmö department to investigate the outcome of patch testing with 2 textile dye mixes (TDMs), consisting of the same 8 DDs tested at the concentration 6.6% and 8.0%, respectively, in Swedish centres representing the Swedish Contact Dermatitis Research Group. The results will contribute to the decision whether a mix of DDs qualifies for inclusion into the Swedish baseline series.

## MATERIAL AND METHODS

### Study population

Seven Swedish dermatology clinics took part in the study from January 1 to December 31, 2011. The participating clinics were from Malmö, Lund, Gothenburg, Uddevalla, Örebro, Stockholm, and Umeå. In these clinics, 2,122 consecutively patch-tested dermatitis patients, 1,424 females (mean age 44.3 years, range 10–94) and 698 males (mean age 44.7 years, range 12–86) took part. The demographic characteristics of the patients are summarised in Table I.

### Substances

The 8 dyes included in the textile dye mix (TDM) 6.6% and 8.0% w/w petrolatum (pet.) were Disperse (D) Blue 35, D Yellow 3, D Orange 1 and 3, D Red 1 and 17, all 1.0% w/w (pet.), and D Blue 106 and D Blue 124, each 0.3% w/w (pet.) in the mix 6.6%, and 1.0% w/w (pet.) in the mix 8.0%, respectively. The dyes were bought from Chemotechnique Diagnostics (Vellinge, Sweden), and the 2 mixes and the separate dye preparations, which were used for the patch testing at the participating clinics, were prepared from the same batches at the Malmö department. All departments except Lund used a baseline series purchased from Chemotechnique Diagnostics. The baseline series included p-phenylenediamine (PPD) 1.0% w/w (pet.) and black rubber mix (BRM) 0.6% w/w (pet.), consisting of 3 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. In Lund, PPD 0.090 mg/cm<sup>2</sup> was tested as a part of the baseline series from Mekostest (Vitaflor Scandinavia AB, Gothenburg, Sweden).

### Patch testing

The test preparations with the TDM 6.6% and 8.0% w/w (pet.), respectively, were provisionally included into the baseline series of the participating dermatology depart-

Table I. Positive reactions to the textile dye mix (TDM), p-phenylenediamine (PPD) and black rubber mix (BRM) when tested simultaneously in 2,212 patients at 7 Swedish dermatology clinics. Simultaneous positive reactions to TDM and PPD in TDM-positive patients and in PPD-positive patients as well as to TDM and BRM in BRM-positive patients, respectively. Positive reactions to the TDM in the patients reacting solely to the TDM but not to PPD nor BRM. The number of patch-tested patients and the proportion (%) are given

Simultaneous positive patch test reactions														
	Total tested	Females %	TDM 8.0 and/or 6.6% positive %	TDM 8.0% positive %	TDM 6.6% positive %	TDM 8.0%/6.6% Ratio	PPD-positive %	BRM-positive %	TDM <sup>a</sup> +PPD/ TDM <sup>a</sup> -positive (%)	TDM <sup>a</sup> +PPD/ PPD-positive (%)	TDM <sup>a</sup> +PPD/ TDM <sup>a</sup> +BRM/ not to PPD nor BRM/ TDM <sup>a</sup> -positive (%)	Reactions to TDM <sup>a</sup> but not to PPD nor BRM/ TDM <sup>a</sup> -positive (%)	Reactions to TDM 8.0% but not to PPD nor BRM/TDM <sup>a</sup> -positive (%)	
Malmö	642	67.3	3.1	3.0	2.5	1.2	2.3	0.6	13/20	13/15	2/4	35.0	42.9	
Lund	295	65.8	2.7	2.7	2.4	1.1	2.0	0	6/8	6/6	0/0	25.0	50.0	
Gothenburg	475	69.7	3.4	3.4	2.7	1.3	3.8	0.6	11/16	12/18	3/3	18.8	33.3	
Uddevalla <sup>b</sup>	165	71.5	3.0	3.0	2.4	1.2	2.4	0.6	2/5	2/4	0/1	60.0	33.3	
Örebro	145	68.3	1.4	0	1.4	0	0.7	0	0/2	0/1	0/0	100.0		
Stockholm	222	61.7	5.4	4.5	4.1	1.1	3.2	1.4	4/12	4/7	1/3	6/12 (50.0)	33.3	
Umeå	178	63.5	0.6	0.6	0.6	1.0	1.1	0	1/1	1/2	0/0			
Total	2,122	67.1	3.0	2.8	2.5	1.1	2.5	0.5	37/64 (57.8)	38/53 (71.7)	6/11 (54.5)	23/64 (36.0)	8/23 (34.8)	

<sup>a</sup>Patients reacting to the textile dye mix (TDM) either at 6.6% or 8.0% or both. <sup>b</sup>IQ Ultra chambers were used at this department.

ments. The patch testing and reading of the patients followed the routine of the clinics. For the patch testing with the TDMs and the 8 individual dyes Finn Chambers® (8 mm diameter; Epitest Ltd, Tuusula, Finland) on Scanpor® tape (Norgesplaster A/S, Vennessla, Norway) were used in all centres except Uddevalla, where IQ Ultra chambers on a high quality hypoallergenic surgical tape (Chemotechnique Diagnostics) were used. The dose for the petrolatum preparations was 20 mg for a Finn Chamber (11) and 25 mg for an IQ chamber. The test chambers were left on the back for 2 days and readings were taken following the guidelines of the International Contact Dermatitis Research Group (12). Reading days were day 3–4 (reading 1) and day 6–8 (reading 2). A dermatologist read all patch tests on both days in all centres except Umeå, where a nurse trained in patch-test readings did the first reading and a dermatologist the second one. Any positive reaction, either on day 3–4 or day 6–8, was registered as a positive reaction in the present study. The patients with positive reactions (+, ++, +++) to at least one concentration of the TDMs at the first patch test reading should be tested with the 8 individual DDs at the same concentrations as in the present mixes. An individual test protocol was filled out for each patient with patch test reactions (allergic, doubtful or irritant) to at least one of the following test preparations: the TDMs, any of the 8 ingredients, PPD or BRM. It was emphasised that all patch test reactions without an obvious morphology of an allergic or irritant nature must be classified as doubtful. An assessment of clinical relevance of the contact allergy to the TDMs was registered in the test protocol in 4/7 patch testing centres (963 patients). This assessment was done by the test-reading dermatologist based on the clinical examination and information provided by the patient on possible exposure to the sensitiser and time course of the dermatitis with regard to the exposure.

#### Statistical analysis

The McNemar test (2-tailed) was used to compare the number of positive reactions to the TDM 8.0% and 6.6%. Fisher's exact test was used to investigate any sex differences in frequencies of positive reactions. We regarded two-sided  $p < 0.05$  as statistically significant.

## RESULTS

The major results are summarised in Table I. Of 2,122 patients 64 (3.0%) reacted to the TDM 8.0% or 6.6%, or both. Contact allergy to the TDM 8.0% was found in 59 patients (2.8%) and to the mix tested at 6.6% in 52 patients (2.5%), i.e. 12 patients were detected only by patch-testing with the TDM 8.0% and 5 patients reacted only to the mix tested at 6.6% (McNemar test  $p = 0.143$ ). Four out of 59 patients (6.8%) positive to the mix at 8.0% only had positive reactions on the second reading. For the mix 6.6% the corresponding result was 3/52 patients (5.8%). Strong reactions (+++/++) were seen in 63% of the positive reactions to the TDM 8.0% and in 75% of the test reactions to the TDM 6.6%. The number of doubtful reactions were 15 (0.7%) and 12 (0.6%) to the mix tested at 8.0% and 6.6%, respectively. Most of these reactions were reported from 2 centres, both having a frequency of positive reactions below 2%. Irritant reactions were found in 2 patients when the TDM was tested at 8.0% and for the mix 6.6%

the corresponding number was 5. More women tested positively to the mix, 3.1% of females versus 2.1% of males for the TDM 8.0% ( $p=0.261$ ) and 2.7% versus 2.0% for the mix tested at 6.6% ( $p>0.3$ ), respectively.

The frequency of contact allergy to the mixes varied between the centres. The range for TDM 8.0% varied between 0–4.5% (Table I). For the mix 6.6% the frequency of TDM-positive patients varied from 0.6 to 4.1%. The ratios between the number of patients found when patch testing with the mix at 8.0% and 6.6% ranged from 0–1.3 at different centres (Table I, Fig. S1<sup>1</sup>).

Contact allergy to PPD was found in 2.5% of the patients (46 females and 7 males;  $p<0.01$ ) and 0.5% of the patients were allergic to BRM (6 females and 5 males;  $p>0.3$ ). Simultaneous contact allergy to PPD was reported in 58% of the TDM-positive patients (Table I). Furthermore, 36% of the TDM-positive patients, i.e. 1.1% of all patch-tested patients, were solely allergic to the TDMs, but not to PPD or BRM (Table I, Fig. S1<sup>1</sup>).

Of the 64 TDM-positive patients 50 subjects were patch-tested with the ingredients. Of these patients 37 (74%) were allergic to at least one separate ingredient when tested at the same concentration as used in the mix. The most frequent single dye allergen in the TDM-positive patients was D Orange 3 followed by D Orange 1. Concomitant reactions to PPD were seen in all but one out of 28 patients allergic to D Orange 3 whereas 2 of the 6 patients allergic to D Blue 106 and/or D Blue 124 also reacted to PPD. These 2 patients also reacted to D Orange 3.

The contact allergy to the TDMs was considered to have a current relevance to the dermatitis in 9 out of the 24 TDM-positive patients (37.5%) where an assessment of clinical relevance was registered in the test protocol and submitted for inclusion in the study.

## DISCUSSION

In the present multicentre study the prevalence of patients with contact allergy to the TDM tested at 2 concentrations in 7 Swedish dermatology departments was evaluated. In the whole study, as well as restricted to the patients at the Malmö department, 2.5% of the patients were allergic to the TDM 6.6%. In a previous study (13), performed in Malmö and in Leuven, Belgium from 2006 until 2008, 2.1% of the Malmö patients were allergic to the TDM patch-tested at the same concentration, 6.6%. These results may indicate that the contact allergy rate to the dye mix is fairly stable or increases, at least in Malmö. Moreover, in the present study, contact allergy to the TDM 8.0% was reported in 2.8% and to the mix tested at 6.6% in 2.5% ( $p=0.143$ ).

However, the proportion of additionally found allergic patients when comparing the mix 8.0% to 6.6% showed 0–30% variation in various departments.

The variation between the participating clinics regarding the frequency of patients having contact allergy to the TDM 8.0% was more than 7-fold whereas the variation was only slightly lower for 6.6% positives. There are many possible explanations for the considerable differences in prevalence of contact allergy to the TDMs between the various centres, including possible differences in referrals of patients for patch testing. Another explanation may be differences in evaluation of the morphology of a test reaction. The majority of the irritant patch test reactions were reported from 2 of the participating clinics, both using Finn Chambers<sup>®</sup>. It is known that several of the DDs used in the TDMs are not only very strong sensitizers but also have a strong irritative potential (14). In the present study, however, the TDM tested at the concentration 8.0% did not give a higher frequency of irritant reactions compared to testing with the mix at the lower concentration.

The percentage of doubtful reactions varied from 0–3.9%. The majority of the doubtful reactions were also reported from 2 centres, both having the lowest frequency of positive reactions to the TDMs. Those centres used Finn Chambers. On the other hand, although the total number of patients tested with IQ chambers was quite low, no statistically significant difference in the frequency of allergic reactions to the TDMs was found between the patients patch-tested with Finn Chambers compared to the patients tested with the IQ test units. The different results from the participating centres, with many doubtful reactions in some centres, may imply that the test reactions to the TDMs sometimes are difficult to read. The fact that the TDM test preparations colour the skin, i.e. gives a bluish tint, may contribute to these results. The variation also implies that standardisation is warranted not only for the dose of the patch test but also for the morphology of irritant, doubtful and weak reactions (15, 16).

Moreover, although the majority of the patients with contact allergy to the TDMs were detected on the first reading, about 6% were only positive on the second reading on days 6–8. These results indicate the importance of a second reading for the TDMs (17) as for several other allergens (18–21).

In the entire study 67% of the patch-tested patients were females. Statistically significantly more women tested positively to PPD. Simultaneous contact allergy to the TDMs and to PPD and/or BRM was found in 64% of the patch-tested patients (Table I). Some patients allergic to the TDMs may initially have been sensitised to PPD, e.g. in hair dyes, or to PPD-related substances such as BRM and then reacted to DDs due to cross-reactivity. They may also have been sensitised by exposure to a common metabolite, rather than DDs

<sup>1</sup><http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1956>



*per se* in textiles. In a previous study (17) a significant association was seen in females regarding contact allergy to PPD and self-reported skin problems arising from synthetic textile materials. As PPD itself is not used as a dye in textiles, the positive reactions to PPD must signify that PPD is a marker of textile dye allergy without being the actual allergen in textiles.

Of all the patch-tested patients 1.1% were allergic to the TDMs but not to PPD or BRM (Table I, Fig. S1<sup>1</sup>). These patients would not have been found if they had only been patch-tested with the Swedish baseline series, which includes PPD and BRM, but not with the mix. TDM-positive patients reacting to D Blue 106 and D Blue 124 more seldom reacted to PPD compared to the patients reacting to the remaining DDs in the mix. It is very important to find patients allergic to D Blue 106 and D Blue 124 as these dyes are regarded as strong sensitizers often giving strong allergic reactions (22, 23).

In the entire study, 74% of the TDM-positive patients who were tested with the ingredients were allergic to at least one separate ingredient when tested at the same concentration as used in the mix. One possible explanation as to why the ingredient testing was negative in some TDM-positive patients could be that the penetration into 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 (24–26).

The most frequent single dye allergen in the TDM-positive patients in the present study was D Orange 3 followed by D Orange 1. In several other studies, however, D Blue 106 and D Blue 124 have been described as common allergens (4, 27) and many authors of studies on contact allergy to DDs have recommended them as screening allergens for textile dye dermatitis. In these studies D Blue 106 and D Blue 124 were patch-tested with the concentration 1.0% (pet.) each (4, 7, 27), the same concentration used in the mix 8.0% in the present study. Due to their strong allergenic potential they have previously been patch-tested at 0.3% w/w (pet.) each in studies initiated by the Malmö department (10, 13). In the present study they were included at different concentrations in the mix 6.6% compared to the mix 8.0% to evaluate if significantly more patients with contact allergy were found when patch-tested with the mix at the higher concentration, 1.0%. D Blue 106 and D Blue 124 tested at this higher concentration revealed 4 additional patients with contact allergy to D Blue 106 and 1 additional patient allergic to D Blue 124, respectively. The results raise the question: Which is the optimal patch test concentration for the ingredients in the mix? Generally, the higher the patch test concentration used, the more individual cases of contact allergy to the dyes will be traced. However, a

higher patch test concentration has a higher risk for adverse effects, including the risk of patch test sensitisation. 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 (28, 29). This must also be considered when deciding the optimal concentration for these 2 blue dyes to be used in a TDM.

The composition of the TDMs was identical to a mix used earlier by Dr Francisco Brandão, Almada, Portugal. This mix has been used in previous studies performed by the Malmö department. In the present study 27 of the 28 TDM-positive patients who were allergic to D Orange 3 also reacted to PPD in the baseline series. Hence D Orange 3 may perhaps be excluded from the mix in the future but this would need further studies.

An allergic patch-test reaction does not necessarily imply a clinically relevant contact allergy. Unfortunately, assessment of clinical relevance was only recorded by the test-reading dermatologist at 4 out of 7 centres. Furthermore, no registration of the sites of dermatitis or the severity of the skin problems was done in the present study. However, according to the assessment the contact allergy to the TDMs was related to the dermatitis in more than 35% of the TDM-positive patients where an assessment of clinical relevance was registered.

According to a study published in 2012, the 8 DDs used in the TDMs seem to be rarely used in textiles today (30), even if they are still present in some European clothes (31). However, it is possible that many of the dyes demonstrated but not chemically identified in the study from 2012 may be contact sensitizers cross-reacting with the DDs in the TDMs used in the present study.

## Conclusions

Patch testing solely with the Swedish baseline series (including PPD) would have missed more than 30% of the patients allergic to the textile dyes as 1.1% of all the patch-tested patients were allergic to the TDMs but not to PPD or BRM. The contact allergy to the TDMs was interpreted as clinically relevant in about 35% of the patients where clinical relevance was registered in the study protocols. The TDM tested at the concentration 8.0% traced more individual cases of contact allergy to the dyes without giving a higher frequency of irritant reactions and other adverse reactions compared to testing with the mix 6.6%. The European Society of Contact Dermatitis recommends a sensitizer for inclusion in the baseline series when routine testing of patients with suspected contact dermatitis results in a contact allergy rate exceeding 0.5–1.0% (15). Therefore inclusion of the TDM, either at 8.0% or 6.6%, into the Swedish baseline series should be considered. Inclusion of the TDM 6.6% into the European baseline series will be suggested this year.

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