

Patch Testing with a Textile Dye Mix and Its Constituents in a Baseline Series

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Background: Among the textile dyes, disperse dyes are common sensitizers.

Objective: To investigate whether patch testing with a textile dye mix consisting of eight disperse dyes would be equivalent to testing with the separate ingredients of the mix at the concentrations used in the mix.

Methods: Researchers tested 1,780 consecutive patients with a mix consisting of Disperse Blue 35, Disperse Yellow 3, Disperse Orange 1, Disperse Orange 31 (misabeled as Disperse Orange 3), Disperse Red 1 and 17, all at 0.5%, and Disperse Blue 106 and 124, both at 0.1%, and with the ingredients at these concentrations. Testing with the labeled dyes at 1.0% was done on 500 of the patients and additionally on the remaining patients who reacted positively to the mix, any of the ingredients, p-phenylenediamine, or black rubber mix.

Results: Thirty-five patients (2%) reacted to the mix, and 34 patients were allergic to at least one ingredient tested at the lower concentration.

Conclusion: The textile dye mix was as good a detector of contact allergy to the disperse dyes as was testing with any combination of the ingredients at the concentration in the mix. Increasing the concentration of the ingredients of the textile dye mix might increase the sensitivity of the mix.

AMONG TEXTILE DYES, disperse dyes (which are used for coloring synthetic textile fibers) are well-known sensitizers.¹ Various studies in consecutively patch-tested dermatitis patients have shown a frequency of contact allergy to separate disperse dyes that ranges from 0.2 to 3.6%.¹⁻⁴ Disperse dyes are not, however, included in most of the commercially available baseline patch test series,⁵ in contrast to p-phenylenediamine (PPD), which historically has been considered to be a screening allergen for textile dye dermatitis.⁶ In a previous study performed at the Department of Occupational and Environmental Dermatology in Malmö, Sweden, 1.5% of 3,325 consecutively patch-tested patients were found to be allergic to a textile dye mix consisting of the following eight disperse dyes:

Disperse Blue 35, 106, and 124; Disperse Yellow 3; Disperse Orange 1 and 3; and Disperse Red 1 and 17.⁷ In that study, we also found a relationship between contact allergy to the textile dye mix (TDM) on the one hand and contact allergy to PPD and black rubber mix (BRM) on the other. The rate of contact allergy found in that study raised the question of whether a TDM should be included in the baseline series.⁸

Patch testing with a mix may save time and space. However, to replace testing with the separate ingredients, one must know how well the results correspond when one is testing with the mix and with the separate ingredients. The main aim of this study was to investigate whether patch testing with a mix of textile dyes would reveal patients with contact allergy to one or more of the separate ingredients when the patients were patch-tested simultaneously with the mix and with the ingredients in the same vehicle and at the same concentration as used in the mix. A secondary aim was to determine the frequency of allergic reactions to the eight disperse dyes when patients were patch-tested with the dyes at the higher concentrations used in commercially available textile patch test series. A third aim was to investigate whether the patients with contact allergy to the TDM and its ingredients could also be traced by patch testing with PPD and BRM, as they are already included in many of the baseline series.

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Materials and Methods

Study Population

The study population consisted of 1,780 consecutively patch-tested dermatitis patients: 1,280 patients at the Department of Occupational and Environmental Dermatology, Malmö University Hospital, Malmö, Sweden, during 2004 and 2005, and 500 patients at the Department of Dermatology, Katholieke Universiteit, Leuven, Belgium, from March 2004 to November 2005. The demographic data on the patch-tested patients (61.2% women [mean age, 42.7 years; range, 13–94 years] and 38.8% men [mean age, 44.0 years; range, 13–88 years]), showed a good correspondence for the two centers.

Patch-Test Preparations

The baseline series used at the Malmö department was purchased from Chemotechnique Diagnostics (Vellinge, Sweden). It included PPD (1% weight per weight [w/w] in petrolatum [pet]) and a BRM (0.6% w/w pet) consisting of N,N'-diphenyl-1,4-phenylenediamine, N-cyclohexyl-N'-phenyl-1,4-phenylenediamine, and N-isopropyl-N'-phenyl-1,4-phenylenediamine, each at 0.2% w/w pet. The department in Leuven bought the baseline series, including PPD (1% w/w pet), from TROLAB (Reinbek, Germany). The 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. Both the TDM (3.2% w/w pet)—labeled as consisting of Disperse Blue 35, Disperse Yellow 3, Disperse Orange 1, Disperse Orange 3, Disperse Red 1, and Disperse Red 17 (all at 0.5% w/w, pet) in addition to Disperse Blue 106 and Disperse Blue 124 (both at 0.1% w/w, pet)—and the separate ingredients at the concentrations used in the TDM were temporarily included in the baseline series in both Malmö and Leuven. The composition was identical to that of a mix used earlier by Dr. Francisco Brandão in Almada, Portugal (personal communication, November 1998). The eight dyes included in the TDM were bought from Chemotechnique Diagnostics, and both the mix and the separate dye preparations were prepared at the Malmö department from the same batches. However, chemical investigations of the eight disperse dyes used at the Malmö department when the study was concluded revealed that the Disperse Orange 3 that was tested at the concentration used in the TDM was actually Disperse Orange 31. The other disperse dyes were as labeled.⁹ The eight disperse dye patch-test preparations (each at 1.0% w/w pet), which were used for patch testing at

the higher concentration both in Leuven and in Malmö, were also purchased from Chemotechnique Diagnostics.

Patch-Test Technique

The patch testing of the patients followed the routines of the two departments. In Malmö, Finn Chambers (8 mm in diameter) (Epitest Ltd Oy, Tuusula, Finland) on Scanpor tape (Norgesplaster A/S, Vennesla, Norway) were used for the patch tests. In Leuven, van der Bend Square Chambers (van der Bend BV, Brielle, the Netherlands) were applied to the patients' backs with 3M Micropore Surgical Tape (3M Health Care, Neuss, Germany) and fixed with Mefix (Mölnlycke Health Care, Göteborg, Sweden). The test chambers were left on the patients' backs for 2 days, and readings were taken according to the guidelines of the International Contact Dermatitis Group.¹⁰ Owing to the different routines at the participating departments, readings were performed on day 3 or 4 and on day 7 or 8 in Malmö and on days 2 and 4 in Leuven. The patch-test reactions on day 3 or 4 were used for registration in the present study.

In Leuven, tests with (1) the TDM, (2) its ingredients at the lower concentration used in the TDM, and (3) its ingredients at the higher concentration were done all at the same time. In Malmö, the patients who had positive reactions to at least one of the following test preparations—BRM, PPD, the TDM, and any of the eight ingredients of the TDM—at the first patch-test reading were then tested with the eight disperse dyes at the higher concentration. The logistics of the study, including the numbers of patients who participated at the two departments, are shown in Figure 1.

Statistical Analysis

The results were analyzed with SPSS version 12.0 (SPSS Inc., Chicago, IL). The Fisher exact test or the McNemar test was used, and we considered two-sided $p < .05$ to be statistically significant.

Results

Consecutive Patch Testing with the TDM and Its Ingredients at Mix Concentrations in the Leuven and Malmö Baseline Series

Contact allergy to the TDM was found in 35 (2%) of the 1,780 patients. Of these 35 positive patients, 25 (71%) were

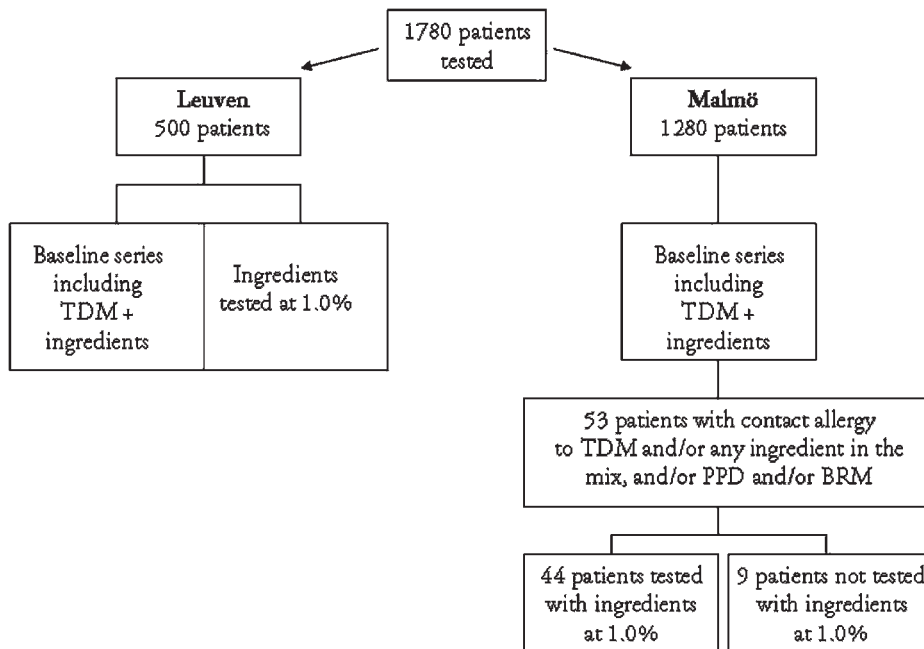


Figure 1. The logistics of the study, including the numbers of patients who participated at the two departments. (BRM = black rubber mix; PPD = p-phenylenediamine; TDM = textile dye mix.)

allergic to at least one ingredient, whereas testing with the TDM missed 9 (26%) of the patients with contact allergy (25 of 35 vs 9 of 1,745; $p < .001$, Fisher exact test, two-sided) (Fig 2 and Fig 3). Thus, 34 patients reacted to at least one ingredient in the mix (Fig 4). The most frequent disperse dye contact allergy in the TDM-positive patients was to Disperse Orange 1, followed by Disperse Yellow 3 (see Fig 3). Simultaneous contact allergies to Disperse Blue 106 and Disperse Blue 124 were found in 6 of 8 patients.

Of the 1,780 patients who were patch-tested, 3.9% were allergic to PPD and 1.0% reacted to BRM. Statistically highly significant associations were found between contact allergy to the TDM and to PPD, between contact allergy to the TDM and to BRM, and between contact allergy to PPD and to BRM ($p < .001$ in each case).

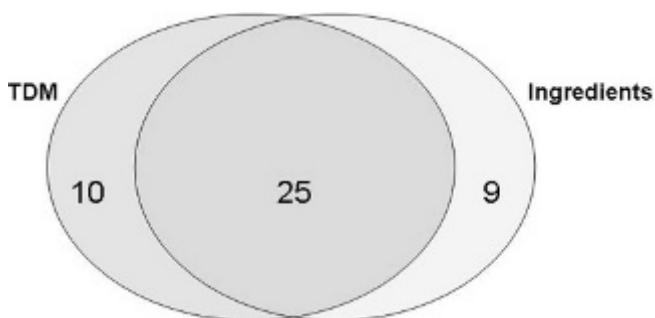


Figure 2. Venn diagram of the numbers of patients (of 1,780 patients) who had positive reactions to the textile dye mix (TDM) and/or to at least one ingredient of the mix tested at the corresponding concentration.

Patch Testing with the Eight Disperse Dyes at Two Concentrations in the Leuven Baseline Series

All 500 patients in Leuven were simultaneously patch-tested with the TDM and with the separate TDM ingredients both at the same concentrations as in the TDM and at the higher concentration (1.0% w/w pet). Contact allergy to at least one disperse dye at the higher concentration was found in 12 of the 13 patients who were allergic to at least one ingredient at the lower concentration and in 16 of the 487 patients with no contact allergy to any ingredient at the lower concentration (12 of 13 vs 16 of 487; $p < .001$, Fisher exact test, two-sided). Contact allergy to Disperse Blue 106 and Disperse Blue 124 on the one hand and Disperse Orange 3 on the other was registered in 3 patients and 2 patients, respectively, among the patients without any contact allergy to the TDM, to the TDM ingredients tested at the lower concentration, to PPD, or to BRM.

Patch Testing with the Eight Disperse Dyes at the Higher Concentration in Leuven and Malmö

The Malmö patients who reacted on day 3 or day 4 to at least one of the following test preparations—PPD, BRM, the TDM, or any of the TDM ingredients—were also tested with the eight dyes at the higher concentration. However, two Malmö patients who had +++ reactions to ingredients tested at the lower concentration were not retested with the ingredients at the higher concentration, but they were still regarded as allergic to the respective dye

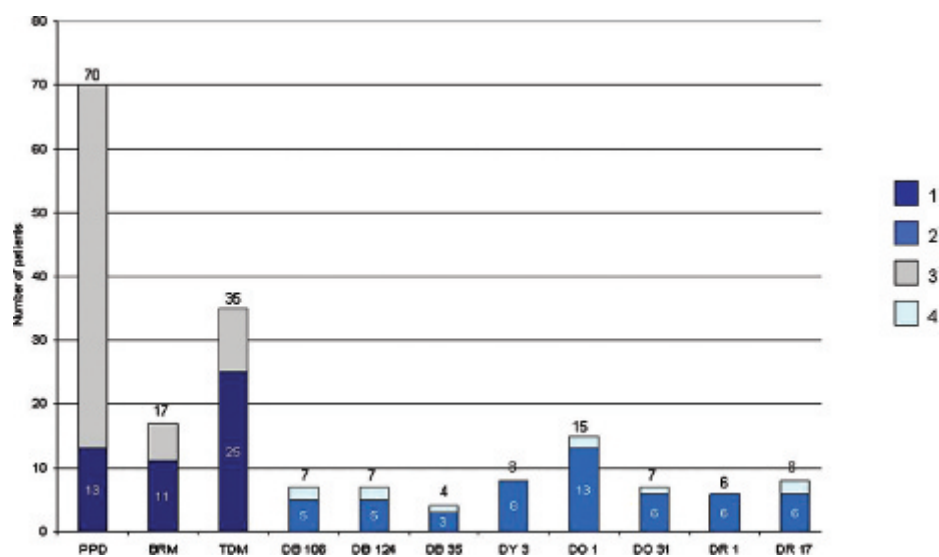


Figure 3. The numbers of patients (of 1,780 patients) who had positive reactions to the textile dye mix, to its ingredients tested at the same concentration, to p-phenylenediamine, or to black rubber mix. The color coding as shown in the right margin is as follows: 1 = the number of patients who had positive reactions to the textile dye mix, p-phenylenediamine, and black rubber mix, respectively, who also reacted to at least one ingredient of the mix; 2 = the number of patients who reacted to the separate dyes and to the textile dye mix; 3 = the number of patients with contact allergy to the textile dye mix, p-phenylenediamine, and black rubber mix, respectively, but who did not react to any ingredients in the mix; and 4 = the number of patients who reacted to the separate dyes without showing contact allergy to the textile dye mix. (BRM = black rubber mix; DB = Disperse Blue; DO = Disperse Orange; DR = Disperse Red; DY = Disperse Yellow; PPD = p-phenylenediamine; TDM = textile dye mix.)

tested at the higher concentration as well. As all patients in Leuven were patch-tested with the ingredients at two concentrations, the data from the Leuven patients'

reactions to these allergens could also be used to investigate the frequency of contact allergy to the eight dyes patch-tested at the higher concentration.

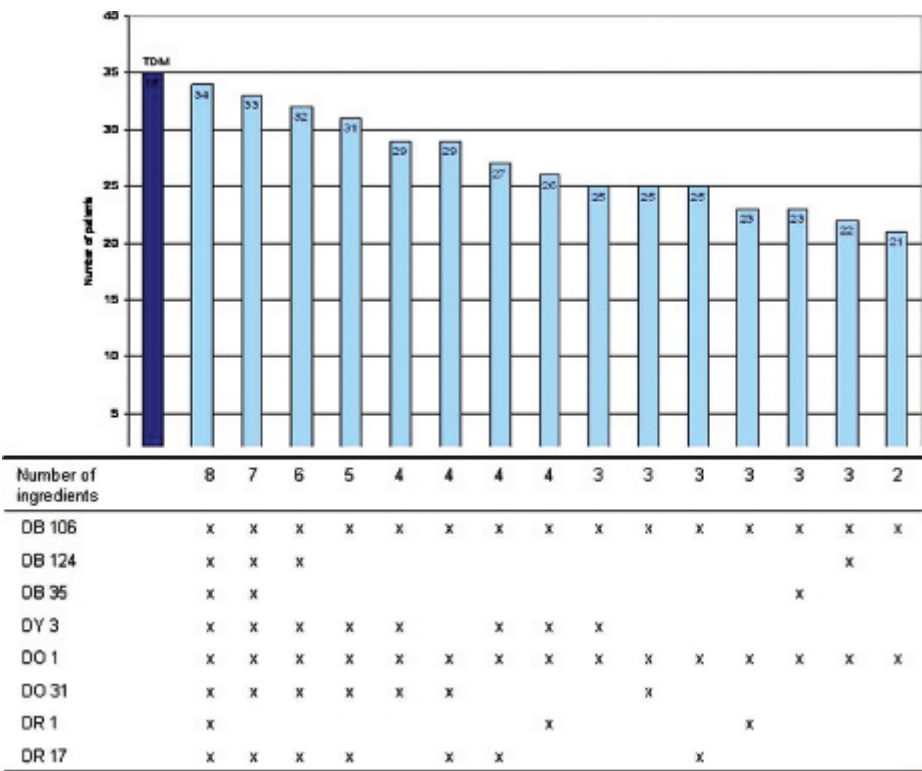


Figure 4. The numbers of patients with contact allergy to the textile dye mix and for combinations of two to eight ingredients giving the highest number of reactors when tested at the concentration in the mix. Each test preparation included in the combination is marked with an x. (DB = Disperse Blue; DO = Disperse Orange; DR = Disperse Red; DY = Disperse Yellow; TDM = textile dye mix.)

Contact allergy to at least one of the following test preparations—PPD, BRM, the TDM, or any of the eight TDM ingredients tested at the same concentration—was found in 100 of the 1,780 patients. Patch testing with the ingredients at the higher concentration was done on 91 of these 100 patients, whereas 9 patients (3 of whom were positive for the TDM) declined to be further tested. The results are shown in Figure 5. Of the 35 patients who reacted to the TDM, 32 were also patch-tested with the TDM ingredients at the higher concentration, and 29 (91%) of these 32 patients reacted to at least one ingredient irrespective of the concentration used for testing. Contact allergy to at least one disperse dye tested at the higher concentration was found in 43 patients: 26 of the 33 patients who were allergic to at least one ingredient at the lower concentration and 17 of the 58 patients who showed no contact allergy to any of the ingredients at the lower concentration (26 of 33 vs 17 of 58; $p < .001$, Fisher exact test, two-sided). For the individual dyes tested at 1.0% w/w, 3 to 22 positive reactions were found, depending on the dye; the highest numbers were for Disperse Orange 3, followed by Disperse Blue 106 and Disperse Blue 124 (see Fig 5). Two patients reacted to both Disperse Orange 31 0.5% w/w and Disperse Orange 3 tested at 1.0% w/w whereas 20 patients were allergic only to the latter preparation.

Discussion

To trace patients with contact allergy to textile dyes, several disperse dye mixes of varying composition have been used for

patch testing in different studies.^{11–15} One common conclusion of these studies was that further investigations are needed, both with regard to which dyes should be included and with regard to the concentrations of the individual ingredients, before deciding on whether a particular dye mix should be added to the baseline series. The main aim of the present study was to investigate the concordance in results between patch testing with a mix consisting of eight disperse dyes and simultaneous patch testing with the separate ingredients of the mix at the same individual concentrations. To obtain more patients, the study was performed at two departments, those in Malmö and Leuven. Because the patch testing followed the routines of each department, Finn Chambers were used in Malmö and van der Bend Square Chambers were used in Leuven. However, according to our experience of using both techniques in parallel at the same department, they give equivalent results.

In the present study, patch testing with the TDM detected as many patients with contact allergy as did the simultaneous patch testing with all eight ingredients separately at the same concentrations (see Fig 4), but only 71% of the TDM-positive patients reacted to any of the separate ingredients of the mix. A possible explanation is that the mix ingredients' penetration of the skin was different when the ingredients were tested separately. Another possible explanation is a compound allergy (additive or synergistic effects of the different allergens) as has been demonstrated when testing with other mixes (eg, fragrance mix).^{16,17} The fact that 5 of the 8 TDM-positive patients without contact allergy to any of the separate ingredients tested at the lower concentration were

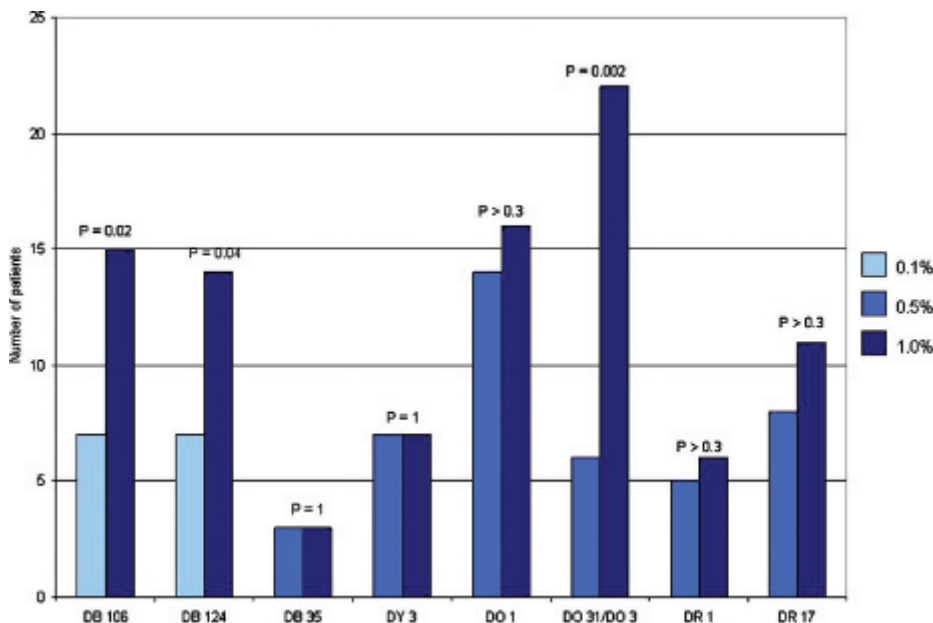


Figure 5. The numbers of patients with positive patch-test reactions to the ingredients of the textile dye mix at the concentrations found in the mix (0.1% or 0.5% weight per weight [w/w] in petrolatum [pet]) and to the corresponding dyes tested at 1.0% w/w pet, among 91 patients patch-tested with the eight dyes. The p values (McNemar test) refer to comparisons between the numbers of patients who had positive reactions to the eight ingredients at each of the two concentrations. (DB = Disperse Blue; DO = Disperse Orange; DR = Disperse Red; DY = Disperse Yellow.)

allergic to at least one dye when it was tested at the higher concentration supports the idea of additive or synergistic effects of the different allergens in the TDM. However, besides the aforementioned physical, chemical, and immunologic aspects, technical aspects may significantly contribute to the variation because it is known that the same patch test may yield different results. This may be one explanation for the fact that nine patients who were allergic to at least one ingredient tested at the lower concentration would have been missed if they had been patch-tested only with the TDM. To trace these nine patients, simultaneous testing with six of the ingredients would have been needed.

In contrast to several other studies reporting Disperse Blue 106 and Disperse Blue 124 to be the most common allergens among the eight dyes tested,^{4,17} Disperse Orange 1 as tested at the lower concentration was the most frequent allergen in our study (see Fig 3). We have been unable to find any reports on patch testing with Disperse Orange 1 in consecutive dermatitis patients, whereas many authors of studies on contact allergy to disperse dyes have consistently emphasized Disperse Blue 106 and Disperse Blue 124 as the dyes of most interest and have recommended them as screening allergens for textile dye dermatitis.^{14,18} One possible reason for the divergent results is that the concentrations of Disperse Blue 106 and Disperse Blue 124 used in other studies were higher than the concentration used in our study (0.1% w/w). Accordingly, simultaneously patch testing with Disperse Blue 106 and Disperse Blue 124 at a 10-times higher concentration in Leuven respectively indicated 5 and 4.5 times as many patients, with contact allergy when compared with the results of patch testing with the dyes at the lower concentration. Although most of these additional patients reacted to the TDM, three Belgian patients would have been missed if they had been patch-tested with only the mix or with Disperse Blue 106 and Disperse Blue 124 at the lower concentration.

Apart from the effect of patch testing with different concentrations, another explanation might be that different batches could have been used for preparing the patch-test preparations. The concentrations of the chemically defined dyes and the presence and concentrations of impurities may vary significantly between different batches as was demonstrated in a previous study at our department in Malmö⁹ (presented in Berlin 2006¹⁹ and later confirmed by a study from Germany²⁰).

No association was found between contact allergy to the "Disperse Orange 3" in the mix and allergy to the corresponding preparation tested at the higher concentration. This puzzling result was explained when the

preparation used in the mix was chemically analyzed with thin-layer chromatography, high-performance liquid chromatography, and mass spectrometry at the Malmö department. The analyses revealed that the test preparation contained Disperse Orange 31 instead (unpublished results). This phenomenon was first described by Le Coz and colleagues.²¹ The consequences of patch testing with Disperse Orange 31 in the TDM and Disperse Orange 31 as an ingredient tested separately at the same concentration (whereas Disperse Orange 3 was used for the patch testing at the higher concentration) must be taken into account when evaluating the results. However, the patch-test results, which showed a low prevalence of contact allergy to Disperse Orange 31 tested at 0.5% and a high frequency of contact allergy to Disperse Orange 3 tested at double the concentration (1.0%), indicate that Disperse Orange 3 is a better substance to use in the TDM than is Disperse Orange 31.

PPD has long been considered to be a screening allergen for textile dye dermatitis.⁶ In the present study, a statistically highly significant association was found between contact allergy to Disperse Orange 3 tested at the higher concentration and contact allergy to PPD and also between allergic reactions to Disperse Orange 3 and reactions to BRM ($p < .001$ each), indicating a cross-reactivity or sensitization to a common metabolite. This association has been described in other studies.²²⁻²⁴ A statistically highly significant association was also found between contact allergy to the TDM and contact allergy to PPD and between allergic reactions to the TDM and reactions to BRM. BRM has three components, all chemically related to PPD. In 38% of the patients with contact allergy to PPD, a simultaneous contact allergy to at least one of the eight dyes tested at the lower concentration was found. The corresponding percentage for contact allergy to BRM and to at least one of the ingredients tested at the lower concentration was 32%. Contact allergy to Disperse Blue 106 and Disperse Blue 124, tested at the lower concentration, was found in 24% of patients who had positive reactions to at least one ingredient tested at the lower concentration (see Fig 3) although these two dyes have been suggested as screening allergens for textile dye dermatitis.^{18,25,26} That PPD and BRM together traced 53% of the patients with contact allergy to at least one ingredient in the TDM and that PPD, BRM, and Disperse Blue 106 at the lower concentration together detected 71% are important as both PPD and BRM are included in many of the baseline series. Even though PPD is nowadays considered to be a better screening agent for contact allergy to hair dyes than for allergy to disperse dyes,²³

another study from Malmö and Leuven found that contact allergy to PPD is an important risk factor for self-reported textile-related skin problems.²⁷ However, many of the patients with contact allergy to PPD and BRM have probably not been primarily sensitized via exposure to disperse dyes in textiles but rather by exposure to hair dyes, chemicals in rubber, or other chemically related substances.

Contact allergy to PPD was significantly more frequent in the patients from Belgium than in the Swedish patients ($p < .001$) whereas no statistically significant differences were seen between the number of patients in the two centers who had positive reactions to the TDM, the ingredients of the TDM, or BRM. However, as the aim of this study was to compare the outcomes of patch testing with the TDM and with its separate ingredients, and as the different frequencies of contact allergy to PPD in Leuven and in Malmö were considered to be for reasons other than the different patch-test procedures, the differences in patch-test results between the two departments are not discussed here.

Disperse Blue 106 and Disperse Blue 124 gave the most disparate patch-test results when tested at different concentrations (McNemar test; $p = .021$ and $p = .039$, respectively) (see Fig 5). Patch testing with Disperse Blue 35, Disperse Yellow 3, Disperse Orange 1, Disperse Red 1, and Disperse Red 17 at the double concentration traced from one to six more patients (15–75%) with contact allergy to the individual dyes (see Fig 5), but most of the reactions were weak, and all but one patient reacted also to the TDM.

Only the patch-test reactions that were registered on day 3 or day 4 (the common reading occasion) could be used for registration in the study. The second reading of the Malmö patients on day 7 revealed three more patients with isolated contact allergy to the TDM and four other patients who were allergic to any ingredient tested at the lower concentration. These four patients, however (with the exception of one patient who had a weak reaction to Disperse Blue 35), were also allergic to the TDM on day 3. Even so, the findings support the importance of late readings as has been reported by other studies.^{28,29}

Aalto-Korte and colleagues also reported late reactions (noticed on day 10 or later) to Disperse Orange 1 and Disperse Orange 3 tested at 1% pet in two patients.³⁰ Both patients also had late reactions to PPD, and retesting indicated that they had been actively sensitized to both PPD and the disperse dyes. In the present study, no late reactions after day 7 were registered. However, it is important to consider the risk of active sensitization when

deciding the optimal concentrations of the ingredients of a modified dye mix.^{8,31}

Conclusions

The purpose of patch testing with a mix is to save space and time. In the present study, patch testing with the textile dye mix detected as many patients with contact allergy as did simultaneous patch testing with each TDM ingredient separately at the same concentrations used in the TDM (see Fig 4) although tests with the TDM failed to detect 26% of patients who had positive reactions to at least one ingredient at this concentration (see Fig 3). Patch testing with Disperse Blue 106 and Disperse Blue 124 at the two concentrations (1.0% and 0.1%) gave the greatest difference in the number of allergic reactions to the separate ingredients. (This does not include the contradictory results of patch tests with Disperse Orange 3 that were caused by incorrect labeling of the material purchased). Ongoing studies using a mix with the separate ingredients tested at higher concentrations may yield more information on optimal patch-test concentrations, especially concerning Disperse Blue 106 and Disperse Blue 124, to highlight patients with contact allergy to the eight dyes tested. These results will form the basis for deciding whether a modified mix of textile dyes would qualify for inclusion in the baseline series.

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There are no conflicts of interests for any of the authors.

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