

Current patch test results in consecutive patients with, and chemical analysis of, disperse blue (DB) 106, DB 124, and the mix of DB 106 and 124

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Disperse blue (DB) 106 and 124 are important textile dye allergens. However, the dye raw material is impure, leading to uncertainty regarding the actual patch test (PT) concentration. To examine, (i) the allergen content of previously and currently used DB 106 and 124 and a respective mix, and (ii) the frequency of positive PT reactions to the DB 106/124 mix and to the single compounds in consecutive PT patients. High performance liquid chromatography (HPLC) analysis and purification of DB 106 and 124, respectively. Descriptive analysis of PT data from the Information network of departments of dermatology obtained between January 2003 and December 2005. Retrospectively, 2 batches of the DB 106/124 mix proved to contain an amount of allergen different to the 1 declared (based on information of suppliers of raw material). However, since February 2005, DB 106 and 124, respectively, are available at a reliable concentration of 0.3% petrolatum. In 2005, the prevalence of positive PT reactions to both the mix (0.89%) and the single constituents combined (0.56%) did not qualify them for inclusion in the standard series. Quality control, providing accurate test concentrations of allergens based on technical grade purity raw materials is necessary for valid diagnosis of contact allergy and comparable epidemiological data.

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A number of years ago, the textile dyes disperse blue (DB) 106 and DB 124 started to raise considerable concern regarding their contact sensitization potential (1). Following initial results from the Information Network of Departments of Dermatology (IVDK) (www.ivdk.org) (2), a mix of DB 106 and DB 124 (1% petrolatum) was conceived and added to the 'monitor series' (3) for a trial period, yielding 1.3% positive reactions in consecutive patients at day 3 readings (4). Thus, for a number of reasons qualifying for inclusion into a standard series, the mix DB 106/124 was added to the German Standard Series in November 2002.

Initially the 2 compounds, which are sometimes, but not always, both contained in textile dyes (5, 6), were found to (i) cross-react considerably (6) and (ii) be hard to purify from each other. Hence, patch testing of the above men-

tioned mix was considered adequate. Since October 2004 Hermal has available purified analytical standards for DB 106 and DB 124, respectively. Commercial test preparations of 2 suppliers used at that time were analysed for their content of DB 106 and DB 124 against these references. Based on these analytical data, it was decided to patch test (PT) 2 new test preparations adjusted to a pure dye content of 0.3% DB 106 in pet. and 0.3% DB 124 in pet., instead of the mix. These 2 single compounds were included into the 'monitor series' (3) from July to December 2005, supplementing the German Standard Series in those IVDK centres using the 'monitor series'. Results of the IVDK network with the 3 allergen preparations, along with (retrospective) analytical results of the DB content of different lots of allergen preparations, are presented and discussed in this paper.

Patients and Methods

As the commercially available dyes (DB 106/DB 124, both supplied from Fluka/Sigma-Aldrich) are normally not pure substances a HPLC method was developed to separate the different components as follows:

- DB 106: assay 43%, loss on drying 6%, and sulfated ash 16%.
- DB 124: assay 24%, loss on drying 6%, and sulfated ash 16%.

The analytic was carried out on a reversed phase HPLC column (Machery-Nagel, Nucleosil 120 C18, 125 × 3 mm, 5 µm) with methanol/water (65/35, w/w for DB 106; 80/20, w/w for DB 124) as solvent system. The isocratic separation runs with a flow of 0.5 ml/min, detection wavelength 584 nm and a sample solution of 1 mg/50 ml. For the quantification of the main components purified analytical standards were used. These standards were purified on an isocratic normal phase HPLC. The separation process yielded standard assays >98%. These standards were used to calibrate the concentration of the single substances (0.3% each) for the TROLAB programme since the beginning of 2005, and to analyse the actual content of DB 106, and 124, respectively, in PT material already produced.

A problem in the processing of the dispersed dyes is the variation of the carrier materials. The nature of this is largely undeclared and unknown, and the manufacturers or suppliers are often not willing to provide any information. In case of DB 106/DB 124 only 1 supplier informed us that a material called 'naphthalene sulfonate' was used. Another problem during the purifying of DB 124 is that DB 106 may be formed in the purifying process by cleavage of the acetyl moiety, making it difficult to yield pure DB 124.

The IVDK network, providing surveillance data on contact allergy, has been described elsewhere (7). In the first part of the analysis, the PT and related results of all patients exposed to DB mix 106/124 (1% pet., obtained by Hermal/Trolab, Reinbek) in the standard series between

January 2003 and December 2005 are descriptively analysed, following current guidelines (8). The second part focuses on the period from July to December 2005, during which DB 106 and DB 124, respectively, each at 0.3% in pet., obtained by Hermal/Trolab, Reinbek, were patch tested in consecutive patients, included in the 'monitor series' (3) by a subgroup of IVDK centres. As standard outcome, the PT reaction 3 days after the start of application (D3) is used. If no D3 reading was performed, the subsequent D4 reading was used instead. For data management and analysis, the statistical software SASTM (version 9.1, SAS Institute, Cary, NC, USA) was used.

Results

The chemical analysis of different lots of DB allergen preparations showed that the allergen content of the DB mix varied considerably between the 2 lots (Table 1); analytical results performed on samples obtained by another supplier of PT material were largely similar and reflected typical contents of DB 106 and 124, respectively, found in the raw material.

In the 3 years of the study period, a total of 24980 patients was patch tested with DB mix 106/124 1% pet., lots 'A' or 'B'. Overall, there were 337 positive reactions (1.35%, exact 95% confidence interval (CI): 1.21–1.50%); $n = 246+$, $n = 64++$ and $n = 27+++$. Doubtful reactions were noted in 1.18% of patients, and irritant reactions in 0.27%, yielding a reaction index of -0.04 . In 6.2% of the patients testing positive to DB mix 106/124 textiles had been suspected as potential cause of current contact dermatitis prior to patch testing, while this proportion was 3.6% in those patients testing negative (Odds Ratio 1.79, exact 95% CI: 1.09–2.80).

In view of the differences between lots 'A' and 'B', test results were stratified for year of PT. In 2003, when only 'A' was used, 128 of 7758 patients (1.65%) tested positive. In 2004, when both lots were in use, these figures were 137 of 9112 patients (1.50%), while in 2005, when 'B' was

Table 1. Actual concentration of DB 106 and 124, respectively, as determined by HPLC analysis (Patients and Methods). All allergens in pet. Estimates for 'use' consider the maximum remaining shelf life

Allergen (nominal concentration)	Supplied	Used	Actual concentration	
			DB 106%	DB 124%
DB mix (1%) 'A'	Until January 2004	Until January 2005	0.02	0.7
DB mix (1%) 'B'	January 2004 to November 2005	January 2004 to November 2006	0.35	0.20
DB 106 (0.3%)	Since February 2005	Since February 2005	0.3	–
DB 124 (0.3%)	Since February 2005	Since February 2005	–	0.3

DB, disperse blue; pet., petrolatum.

(predominantly) used, the proportion of positive patients was only 72 of 8110 (0.89%). The difference between the years was significant, according to the results of a logistic regression analysis, considering age as confounder ($P < 0.0001$, Wald χ^2).

Results with the 2 single compounds and the mix obtained in the subset of patients patch tested with the 'monitor series' in the second half of the year 2005 are presented in Table 2. Positive reactions to DB 106 were observed in 11 of 2215 patients tested (0.50%, exact 95% CI: 0.25–0.89%). The respective figures for DB 124 were 8 positive reactions of 2214 patients tested (0.36%, exact 95% CI: 0.16–0.71%). In the subgroup of patients consecutively tested in the second half of the year 2005, 19 positive reactions to the DB mix 106/124 (lot 'B') were observed (0.74%, exact 95% CI: 0.45–1.16%). It was noted that 5 more reactions turned positive on D4 after being negative or doubtful on D3 in case of DB 106, 1 more in case of DB 124 and 3 more in case of the DB mix. However, as D4 readings are not performed routinely in most departments (if a D3 reading had been carried out), these results pertaining to D4 reactions remain anecdotal and are not considered further. Altogether 2186 patients had been patch tested with each of the 3-allergen preparations. The pattern of cross-reactivity is shown in Table 3.

Discussion

The present analysis provides a current update regarding the frequency of positive test reactions to DB 106 and/or DB 124 since these allergens have gained scientific and medical attention in the 1980's (9, 10). The problem of the impurity of raw materials has been known since the beginning, leading, for example, Hausen and Sawall to employ an extensive chromatographical purification process before being able to perform sensitization experiments with pure DB 124 (10). However, to the best of our knowledge, the problem of the purity of the raw material used to prepare these PT allergens for human use, and, in particular, the actual content of DB 106 and DB 124, respectively, has been addressed by our study

Table 2. Results of patch testing DB 106, DB 124, and the DB mix 106/124 in consecutive patients, July to December 2005^a

	Percent	<i>n</i> _{tested}	?	IR	+	++/+++
DB 106	0.3	2215	13	3	9	2
DB 124	0.3	2214	11	5	6	2
DB mix 106/124	0.35+0.20	2555	19	2	16	3

DB, disperse blue; IR, irritant reaction.

^aAll allergens in petrolatum.

Table 3. Patterns of cross-reactivity between DB 106, DB 124, and DB mix 106/124 'B', respectively, restricted to those patients tested with all 3 compounds ($n = 2186$). Concordance is quantified with Cohen's kappa accompanied by a 95% CI

(a) DB 106 versus DB 124 [$\kappa = 0.63$ (0.38–0.88)]			
DB 124	DB 106	pos.	non-pos.
	pos.	6	2
	non-pos.	5	2173
(b) DB 106 versus DB mix 106/124 'B' [$\kappa = 0.40$ (0.15–0.64)]			
DB 106	DB mix	pos.	non-pos.
	pos.	5	6
	non-pos.	9	2166
(c) DB 124 versus DB mix 106/124 'B' [$\kappa = 0.54$ (0.29–0.79)]			
DB 124	DB mix	pos.	non-pos.
	pos.	6	2
	non-pos.	8	2170
(d) DB 106 or DB 124 versus DB mix 106/124 'B' [$\kappa = 0.44$ (0.21–0.68)]			
DB 106 or 124	DB mix	pos.	non-pos.
	pos.	6	7
	non-pos.	8	2165

DB, disperse blue; pos., positive; CI, confidence interval.

for the first time. A quantitative analysis has become possible after the availability of both a suitable HPLC methodology, and pure reference substances. The results indicate that the actual allergen content has been for a long time, and in some allergen preparations still is, (much) lower than declared. Alas, this is not an entirely unusual situation if technical grade raw material is used, which is sometimes unavoidable. An aspect still needing further investigation is the 'carrier' substance(s), which remain a yet unidentified part of the allergen preparation, and of the dye mixture used to treat textiles, for that matter.

The quantitative results of chemical analysis have a Janus face: retrospectively, the test results with previously used allergens are put into perspective, regarding the actual test concentration used, and prospectively, a well-defined preparation (at least with regard to concentration and stability of the actual allergen) is available for patch testing. The unintentional imbalance of DB 106 and DB 124 in the mix 'A', used, for instance, in a previous analysis (4) may not have compromised the results of using this mix severely in retrospect, in view of the fairly marked cross-reactivity between DB 106 and DB 124 (Table 3). Notably, the cross-reactivity was now noted between pure compounds – previous analyses of cross-reactivity (2) were probably biased by mutual contamination of the compounds. Regardless of this, immunological cross-reactivity tends to be over-estimated in the clinical setting anyway, as previous co-exposure and concomitant 'immunologically independent' sensitization can never be excluded, or must rather be suspected in case of DB 106 and DB 124 because of mixed exposure via textiles.

The fact that actual allergen concentrations were considerably lower than declared may have had the advantage to contribute to the safety of patch testing with at least DB 106: in the meantime, concerns regarding the risk of active sensitization by DB 106 when patch tested at 1% (pet.) have been raised, based on extremely low EC₃ values noted in 1 laboratory so far, namely, 0.015% (11). This is well in line with a previous judgment of DB 106 being 'the strongest sensitizer found so far among the azo disperse dyes' (12). In comparison, sensitization experiments with purified DB 124 have shown this compound to be only a moderate sensitizer (13). From this background, the current concentration of 0.3% seems more appropriate than an actual (not: nominal) concentration of 1% at least in case of DB 106.

In the most current period analysed, 0.59% (exact 95% and CI: 0.32–1.01%) of the consecutive patients tested reacted positively to either DB 106 or DB 124 or both (Table 3, panel a). Hence, the criterion of 'at least 1% positive reactions in consecutive patients' an allergen has to meet to qualify for inclusion in the standard series is largely not met. The same holds true for the DB mix 'B' patch tested in 2005. Anyway this mix was not considered necessary any more, in view of the availability of its just 2 single compounds. Consequently, these textile dyes have been removed from the German Standard Series, to be tested in a special textile dye series, which includes also a number of other dye allergens [results with this series have been published (14)]. This decision has an important consequence: as the patient's history and the clinical picture of dermatitis are not always suggestive of textile (dye) dermatitis, but may be rather non-specific or mimic atopic or nummular eczema, the dermatologist needs to actively consider applying the textile dye series in cases of doubt. In contrast, the inclusion of an allergen in the standard series, which is applied to virtually every patient patch tested, is more robust against a lack of diagnostic vigilance.

Conclusion

Although the low, technical grade purity of DB 106 and DB 124 has been known for a long time, PT allergens with a strictly controlled concentration have become available only recently, after establishing analytical standards. However, patch testing with such better-defined allergens is just one, albeit essential, building block of adequate diagnostic work-up of (suspected) textile dye allergic contact dermatitis. The clinical relevance of positive reactions to different types of dyes (the 2 disperse dyes analysed here being just 2 of the

more important ones out of a vast range) should be assessed, for example, following an algorithm suggested by Hatch et al. (15). Interestingly, dyes to which patients are positive upon patch testing are not always present in culprit garments, according to chemical analyses (5). Hence, it may be useful to examine those garments further which have clinically caused contact dermatitis and/or have been positive upon patch testing with regard to (i) the nature of fibres, (ii) colour (perspiration) fastness, and (iii) the nature of the dyes contained. Patch testing the patient with a chromatogram of his or her garment [(16), p. 698], where applicable, may not only help to solve an individual diagnostic riddle, but also give interesting clues on yet unknown, or under-estimated contact allergens among the textile dyes.

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