

Chemical investigations of disperse dyes in patch test preparations

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Background: Contact allergy to textile dyes is not uncommon. The allergy is detected by patch testing patients with commercial patch test preparations.

Objective: To investigate 8 disperse dyes (DDs) used for patch testing in the departments in Malmö and in Leuven and to compare them with test preparations used at various dermatology departments.

Materials/Methods: The investigated DDs were Disperse Blue (DB) 35, 106, and 124, Disperse Yellow (DY) 3, Disperse Orange (DO) 1 and 3, and Disperse Red (DR) 1 and 17. From 13 clinics, 107 petrolatum preparations were analysed using high-performance liquid chromatography and thin-layer chromatography (TLC), and compared with reference substances obtained at the Malmö laboratory. Concerning DB 35, no reference substance could be identified.

Results: TLC visualized impurities in all DDs. For each DD, except DB 35, the mean concentration in the preparations labelled to contain 1.0% (w/w) were DB 106: 0.30%, DB 124: 0.25%, DY 3: 0.44%, DO 1: 0.40%, DO 3: 0.68%, DR 1: 0.49%, and DR 17: 0.35%; there were variations between the samples also with regard to the number of impurities. DO 3 could not be demonstrated in 4/15 preparations labelled DO 3.

Conclusion: The results may have implications for individual diagnosis and prevention and when comparing test results from various centres.

Key words: contact allergy; disperse dyes; high-performance liquid chromatography; impurities; patch test preparations; reference substance; thin-layer chromatography. © Blackwell Munksgaard, 2008.

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Contact allergy to textile dyes is a cause of both occupational and nonoccupational skin manifestations (1–4). However, the diagnosis is often missed because of the absence of textile dyes in most standard patch test series or a clinical picture not giving suspicion of textile dermatitis because of contact allergy (1, 2, 4–6). Disperse dyes (DDs) are the most common sensitizers among textile dyes. They are used for colouring mixed and synthetic textile fibres such as acetate, acrylics, polyamide, and polyester fibres. Many DDs known to cause contact allergy are included in commercially available textile patch test screening series. Suppliers of such patch test preparations include Chemotechnique Diagnostics (Vellinge, Sweden),

Hermal (Trolab) (Reinbek, Germany), FIRMA (Florence, Italy), and Martí Tor (Barcelona, Spain).

To investigate the prevalence of contact allergy to different textile dyes in patients patch tested at the Department of Occupational and Environmental Dermatology (University Hospital, Malmö, Sweden), a textile dye mix (TDM) was included in the standard test series used at the department from 1999 to 2005. The TDM was also used for patch testing at the Department of Dermatology (Contact Allergy Unit, University Hospital, K.U. Leuven, Belgium) from 2004 to 2005. The mix consisted of 8 DDs, i.e. Disperse Blue (DB) 35, Disperse Yellow (DY) 3, Disperse

Orange (DO) 1 and 3, Disperse Red (DR) 1 and 17, all 0.5% (w/w) petrolatum (pet.), in addition to DB 106 and 124, both 0.1% (w/w) pet. Chemical structures of the 8 DDs are shown in Fig. 1.

1.5% of 3325 consecutively tested patients in Malmö from 1999 to 2003 reacted positively to the TDM (7). The high contact allergy rate found in the study raised the question whether a TDM generally should be included in national and/or international standard patch test series (8). Most of the TDM-positive patients were additionally

tested with the separate dyes included in the mix. The test results concerning the separate components in the TDM (7) varied compared with other published studies. Only 1 patient reacted to DO 3, contrary to a study from Italy (9), where DO 3 and DB 124 gave the highest number of positive test reactions. One explanation for the different test results could be the choice of clothing materials and different ways of dressing in various regions. This explanation is supported by a study from Spain (10) indicating a high prevalence of DD

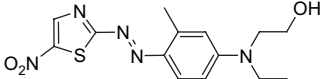
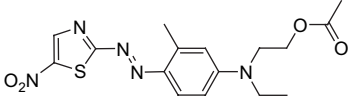
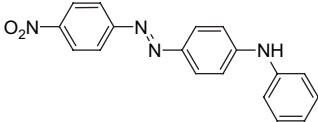
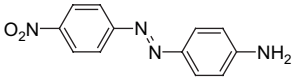
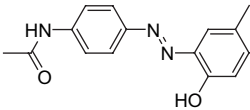
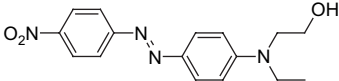
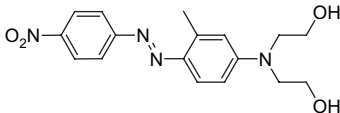
Disperse Blue 35	Chemical formula not specified according to CAS	CAS: 12222-75-2 CI: not found
Disperse Blue 106		CAS: 68516-81-4 CI: 111935 MW: 335
Disperse Blue 124		CAS: 15141-18-1 CI: 111938 MW: 377
Disperse Orange 1		CAS: 2581-69-3 CI: 11080 MW: 318
Disperse Orange 3		CAS: 730-40-5 CI: 11005 MW: 242
Disperse Yellow 3		CAS: 2832-40-8 CI: 11855 MW: 269
Disperse Red 1		CAS: 2872-52-8 CI: 11110 MW: 314
Disperse Red 17		CAS: 3179-89-3 CI: 11210 MW: 344

Fig. 1. Chemical structure, Chemical Abstracts Service (CAS) and Colour Index (CI) numbers as well as molecular weight (MW) for each investigated disperse dye.

allergy with a different prevalence in various areas probably because of social and cultural factors. Another explanation could be impurities present in the patch test preparations used to trace contact allergy, giving qualitative and quantitative differences, although the DDs used for patch testing were labelled with the same concentration. The possibility of cross-sensitivity to the pure dye substance and impurities with chemical similarities, or additive/synergistic effect between the pure dye and the impurities, may also give a higher yield of positive patch test reactions than expected.

The aim of the present study was to chemically investigate the purity and concentration of the 8 DDs in the patch test preparations used in the previous testing at the departments in Malmö (7) and in Leuven and to compare them with test preparations labelled to contain the same DDs used at various dermatology departments around the world.

Materials and Methods

Chemicals

Heptane was obtained from Mallinckrodt Baker B.V. (Deventer, the Netherlands) and chloroform, acetone of analytical grade (AC0311), and acetonitrile of fluorescence high-performance liquid chromatography (HPLC) grade (AC0335) from Scharlau Chemie S.A. (La Jota, Barcelona, Spain). Acetonitrile for the thin-layer chromatography (TLC) system was obtained from Lab-Scan (Dublin, Ireland), triethylamine from Fisher Scientific (Loughborough, UK), and ethanol 99.5% v/v from Kemetyl (Haninge, Sweden). Pet. (Vaseline album, snowwhite USP/NF kval. E) was bought from Apoteket (Göteborg, Sweden). Raw materials for preparing each of the 8 DD patch test preparations used at the departments in Malmö and in Leuven were bought from Chemotechnique Diagnostics. The reference substances for each of the DDs were isolated and identified at the Malmö department (to be published). The purity of the reference substances was >99%, except for DO 3, which had a purity of >97%. We have not been able to isolate and identify any reference substance for DB 35. The chemical structures, Chemical Abstract Service (CAS), and Colour Index (CI) numbers as well as the molecular weights of the identified DDs are shown in Fig. 1 (11, 12).

Patch test preparations

To acquire a general view of the status of the Pet. patch test preparations containing the 8 DDs, we contacted 19 dermatology departments in various parts of the world and asked them whether they

would send us the DD test preparations they presently used for patch testing. We obtained a positive reply from 12 of them, 7 European, 3 North American, 1 Asian, and 1 Australian department, and the preparations were delivered from April to August 2005. The preparations were made by 4 commercial companies representing 4 countries. More than 80% of the preparations came from 1 supplier. 1 preparation, containing DB 106 and labelled 0.1%, was not marked with the supplier. In addition to the patch test preparations from the various departments, we also obtained patch test preparations of DB 124, DY 3, DO 3, DR 1, and DR 17 directly from 1 of the suppliers. Patch test preparations and suppliers used by the various departments, as well as labelled concentrations on each syringe, are found in Table 1.

Preparations purchased from supplier 1 after 1994 were labelled 1.0% pet., whereas older preparations from supplier 1 and all preparations from suppliers 2 and 4, respectively, were labelled 1% pet. The patch test preparations from supplier 3 lacked information of the concentration. The test preparations used for patch testing at the departments in Malmö and in Leuven were prepared at the Malmö laboratory. A total of 107 test preparations from 13 departments were analyzed from December 2005 to August 2006. The syringes were stored in a refrigerator from arrival until analysis. The number of preparations that had passed the expiry date before their arrival to the laboratory as well as the number of preparations that expired during the investigative period are shown in Table 1.

High-performance liquid chromatography

A ThermoFinnigan system was used consisting of a P4000 quaternary pump, a ultraviolet (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 i.d. × 250 mm) was packed with Kromasil, 100 Å, 5 µm (Eka Nobel, Bohus, Sweden). The detector scanned the eluate in the range 200 to 800 nm. Solvents A and B consisted of acetonitrile/water 40/60 v/v and acetonitrile, respectively. The linear gradient elutions used for the different dyes are described in Table 2. The concentration of the DD in the investigated patch test preparations was determined by comparing the peak area produced by a known concentration of the DD reference substance with the peak area of the corresponding peak from the sample. Identity of the substance producing a

Table 1. The disperse dye patch test preparations used by the various departments. Suppliers, stated and detected concentrations in each analyzed test preparation

Dep.	Supp.	Stated conc. (% w/w)	Detected conc. (% w/w)	CV (%)	Expired	Supp.	Stated conc. (% w/w)	Detected conc. (% w/w)	CV (%)	Expired
Disperse Blue 106						Disperse Blue 124				
A	1	1.0	0.30	18.8		1	1.0	0.24	25.3	
B	1	1.0	0.32	14.6	*	1	1.0	0.25	15.4	†
C	nd	0.1	0.04‡	14.4	†	1	1.0	0.23	6.6	†
D	1	1.0	0.32	26.5		1	1.0	0.23	§	
E	1	1.0	0.27	22.5	*	1	1.0	0.25	6.9	*
F	1	nd	0.33	9.8		3	nd	0.18	6.5	
G	1	1.0	0.36	11.1	*	1	1.0	0.26	13.3	*
H	1	1.0	0.34	10.2	*	1	1.0	0.23	11.5	†
I	1	1.0	0.33	12.4	*	1	1.0	0.34	§	*
J	1	1.0	0.32	26.9	*	1	1.0	0.25	14.4	*
K	1	1.0	0.13	22.8	*	1	1.0	0.24	8.8	*
L	1	1.0	0.33	18.4	*	1	1.0	0.27	7.4	†
M	**	1.0	0.29	27.4		**	1.0	0.24	7.2	
—		—	—	—	—	4	1	0.26	4.4	
Mean conc. of detected dye			0.30					0.25		
Disperse Blue 35						Disperse Yellow 3				
A	1	1.0				1	1.0	0.50	18.0	
B	1	1			*	1	1	0.39	6.4	*
C	1	1.0			†	3	1	0.44	4.8	†
D	1	1.0			†	1	1.0	0.43	0	
E	1	1			*	1	1.0	0.41	5.1	*
F	—	—			—	3	nd	0.46	12.1	
G	1	1.0			†	2	1	0.47	7.7	*
H	1	1.0			*	2	1	0.40	5.7	*
I	1	1.0			*	1	1.0	0.49	§	*
J	1	1.0			*	1	1.0	0.27	§	*
K	1	1.0			*	1	1.0	0.40	10.9	*
L	1	1.0			*	1	1.0	0.43	7.0	*
M	**	1.0			—	**	1.0	0.61	43.5	
—		—			—	4	1	0.48	14.0	
Mean conc. of detected dye								0.44		
Disperse Orange 1						Disperse Orange 3				
A	1	1.0	0.46	35.9		1	1.0	0.88	4.0	
B	1	1	0.39	21.9	*	1	1	0.25	4.0	*
C	1	1.0	0.36	13.6	†	1	1.0	0.77	1.5	
D:a	1	1.0	0.34	16.4		1	1.0	0.85	11.2	
D:b	—	—	—	—	—	2	1	0.80	5.6	
E	1	1.0	0.36	20.3	*	1	1.0	<0.009		*
F	—	—	—	—	—	3	nd	0.38	4.1	†
G	1	1.0	0.35	24.7	*	2	1	0.79	8.2	*
H	1	1.0	0.42	17.7	*	2	1	0.92	3.8	*
I	1	1.0	0.57	§	*	1	1.0	<0.009		*
J	1	1.0	0.40	13.2	*	1	1.0	<0.009		*
K	1	1.0	0.36	24.2	*	1	1.0	0.93	4.3	
L	1	1.0	0.39	14.9	*	1	1.0	0.61	0.9	
M	**	1.0	0.37	7.2		**	1.0	<0.009		
—		—	—	—	—	4	1	0.34	4.5	
Mean conc. of detected dye			0.40					0.68		
Disperse Red 1						Disperse Red 17				
A	1	1.0	0.37	31.7		I	1.0	0.35	26.2	
B	1	1	0.39	10.3	*	1	1	0.38	2.6	*
C	1	1.0	0.35	§	†	1	1.0	0.32	3.6	†
D	1	1.0	0.45	§		1	1.0	0.33	6.1	
E	1	1.0	0.37	15.0	*	1	1	0.28	6.2	*
F	3	nd	0.39	11.8		—	—	—	—	—
G	2	1	1.01	15.3	*	2	1	0.40	6.2	*
H	2	1	1.03	17.5	*	2	1	0.34	9.1	*
I	1	1.0	0.43	§	*	1	1.0	0.31	27.9	

(Continued)

Table 1. Continued

Dep.	Supp.	Stated conc. (% w/w)	Detected conc. (% w/w)	CV (%)	Expired	Supp.	Stated conc. (% w/w)	Detected conc. (% w/w)	CV (%)	Expired
J	1	1.0	0.41	21.3	*	1	1.0	0.39	11.8	*
K	1	1.0	0.42	12.4	*	1	1.0	0.38	5.3	*
L	1	1.0	0.39	12.0	*	1	1.0	0.33	10.9	*
M	**	1.0	0.38	5.4		**	1.0	0.27	18.4	
	4	1	0.47	11.3		4	1	0.50	8.7	
Mean conc. of detected dye			0.49					0.35		

Conc., concentration; CV, coefficient of variation for the triplicate samples; Dep., department; nd, not declared; Supp., supplier.

*Expiry date passed at arrival.

†Expiry date passed during the investigative period.

‡Test preparation excluded when mean concentration was calculated.

§Syringes which contained too little test material for triplicate analyses.

**Test preparation made at the Malmö department.

Table 2. The linear gradient elutions used for high-performance liquid chromatography analysis of the disperse dyes*

DO 1, DO 3, DB 106, DB 124			DY 3		DR 1		DR 17	
Time (min)	%B	Flow rate (ml/min)	Time (min)	%B	Time (min)	%B	Time (min)	%B
0	40		0	50	0	52.5	0	35
35	100		7.5	50	7.5	52.5	7.0	100
45	100	2.0	7.6	100	7.6	100	10.0	100
			13.0	100	13.0	100		

DB, Disperse Blue; DO, Disperse Orange; DR, Disperse Red; DY, Disperse Yellow.

*Solvents A and B consisted of acetonitrile/water 40/60 v/v and acetonitrile, respectively. The flow rate was 1.0 ml/min if not otherwise indicated.

certain peak was determined by retention time and UV spectrum.

Work-up procedure

About 0.1–0.5 g of each Pet. preparation was accurately weighed into a test-tube and dissolved in 5.0 ml heptane. By adding 5–6 ml ethanol/acetone/water 45/45/10 (v/v/v), the dye was gradually extracted and separated to the ethanol/acetone/water phase. The procedure was repeated 3–4 times until the heptane phase was colourless. For separation of 4 of the preparations labelled DO 3 to the ethanol/acetone/water phase, more than 20 extraction procedures were needed to make the heptane phase colourless. The respective ethanol/acetone/water solution containing the dye was evaporated to dryness in vacuum. The residue was dissolved in 1.0 ml acetone. The solution was diluted 25–1000 times in mobile phase A prior to HPLC analysis. To evaluate the homogeneity of the patch test preparations, 3 samples from each preparation were made. The extraction of each sample was performed according to the work-up procedure and analyzed using analytical HPLC (Table 2). 8/107 syringes did not contain enough test material for triplicate analyses.

Thin-layer chromatography

50 µl of the acetone solution from the work-up procedure was deposited on the lower part of a sil-

ica gel plate (TLC plates 20 × 20 cm with silica gel 60 F 254 on glass from VWR International, Stockholm, Sweden). The plate was placed in a jar containing an eluent, a mixture of chloroform, and acetonitrile 86/14 (v/v). If the dye consisted of more than 1 chemically defined substance, the eluent carried some components further on the plate than others, giving rise to a band of separate spots. The locations of the spots are described with the ratio of the distance travelled by the centre of the respective spot to the distance simultaneously travelled by the front of the solvent (R_f). The acetone solutions of DB 106 and DB 124 were also analyzed with an eluent containing a mixture of chloroform, acetonitrile, and triethylamine 75/20/5 (v/v/v) to obtain a better separation of the spots (Fig. 3). When the migration was finished, the plate was removed to dry. The thin-layer chromatograms were all inspected in visible light and in UV radiation (254 and 366 nm), and each patch test preparation was compared with the TLC pattern of the corresponding DD reference substance.

Extraction recovery

About 10 mg of each of the 8 commercial DDs, bought from Chemotechnique Diagnostics for preparing the patch test preparations, was accurately weighed and mixed with pet., yielding a 1.0% (w/w) preparation. The whole preparation was extracted according to the work-up

procedure earlier described and was analyzed using analytical HPLC. At least 3 preparations and subsequent extractions were made from each dye to evaluate the reproducibility of the work-up procedure.

Repeatability of the HPLC method

In order to investigate the repeatability of the HPLC method, a sample was taken from a patch test preparation representing each of the DDs, except DB 35. From each sample, performed according to the work-up procedure, triplicate samples of the acetone solutions were diluted and analyzed using analytical HPLC, according to the method earlier described. The analyses showed a coefficient of variation (CV) between 0% and 0.39%.

Results

The DD patch test preparations used by the various departments, the suppliers, stated and detected concentrations, CV for the triplicate samples in each analyzed test preparation, and the test preparations that had past expiry date at arrival or during the investigative period are shown in Table 1. 2 patch test preparations labelled DR 1 contained the stated concentration, whereas all patch test preparations labelled DB 106, DB 124, DY 3, DO 1, and DR 17, 10/15 preparations labelled DO 3, and 12/14 preparations labelled DR 1 contained <80% of the stated concentration. All the patch test preparations showed several peaks when analyzed with HPLC, and the pattern could also be visualized as different spots on the TLC plates (Figs 2–4). The investigations did not indicate any correlation between the expiry date and the concentration, nor did the TLC pattern. Although 7/8 of the investigated DDs were chemically defined, the results demonstrated impurities in all preparations. The TLC patterns described below for each DD are the spots seen in visible light. The thin-layer chromatograms of the collected test preparations all showed 1 additional spot with the same R_f when inspected in UV radiation. These spots had moved further than the coloured spots seen in visible light. 1 spot with the same R_f could be visualized in UV radiation of a TLC plate made from an acetone solution of pure pet. extracted according to the work-up procedure but not on the TLC plates representing the DD reference substances without any contact with pet.

Disperse Blue 106

The average extraction recovery for the work-up procedure was 92.4% (CV 18.1%). The mean

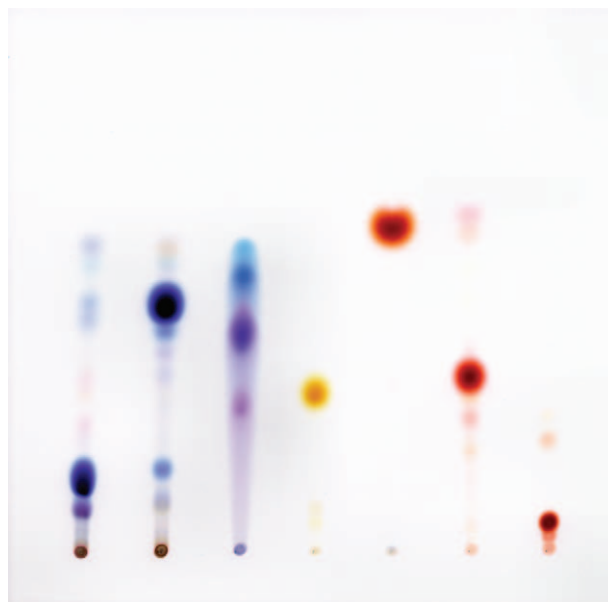


Fig. 2. The thin-layer chromatography patterns of Disperse (D) Blue 106, 124, and 35; D Yellow 3; D Orange 1; D Red 1 and 17 (from left to right).

concentration of DB 106 in the 12 patch test preparations, the syringe labelled 0.1% excluded, was 0.30% (range 0.13–0.36) when analyzed with HPLC (Table 1). The TLC pattern of the acetone solutions of the extracts of the patch test preparations looked fairly the same with 10 defined spots, including 1 intense blue spot with R_f corresponding to the R_f of DB 106 (Figs 2 and 3). The pattern was the same for all the preparations, except for 2 preparations missing a pink spot. 1 of these preparations, labelled 0.1%, showed a pink spot when applied on a TLC plate in 5 times higher concentration. The other preparation, which only contained 0.13% of DB 106, was never tested at a higher concentration. All preparations, except the preparation labelled 0.1%, showed a blue spot with R_f corresponding to the R_f of DB 124 (Fig. 3), and the HPLC analysis also showed a peak corresponding to this dye. The highest possible concentration of DB 124 in the preparations labelled DB 106 1.0% varied from 0.002% to 0.0004% when analyzed with HPLC, thus 1/500 to 1/2500 of the stated DB 106 concentration. The oldest preparation, which expired in 1995, had the same TLC pattern as the preparations not yet expired.

Disperse Blue 124

The average extraction recovery was 88.5% (CV 17.4%). The mean concentration of DB 124 in the 14 patch test preparations was 0.25% (0.18–0.34). The TLC plates showed 11 defined spots, including

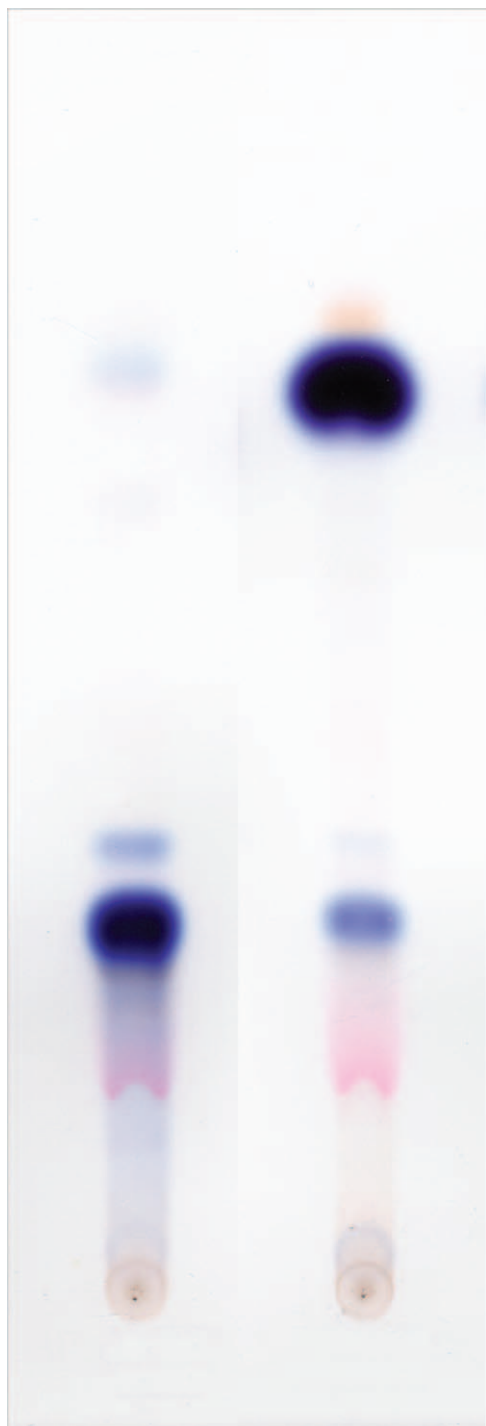


Fig. 3. The thin-layer chromatography patterns of Disperse Blue 106 and 124 (from left to right) when analyzed with an eluent containing a mixture of chloroform, acetonitrile, and triethylamine 75/20/5 (v/v/v).

a marked blue spot with R_f corresponding to DB 124 (Figs 2 and 3). The intensity of the main spot was fairly the same, independent of the concentration of DB 124. The TLC analysis of all the patch test preparations visualized a weaker blue spot with R_f corresponding to the R_f of DB 106 (Fig. 3). The highest possible concentration of

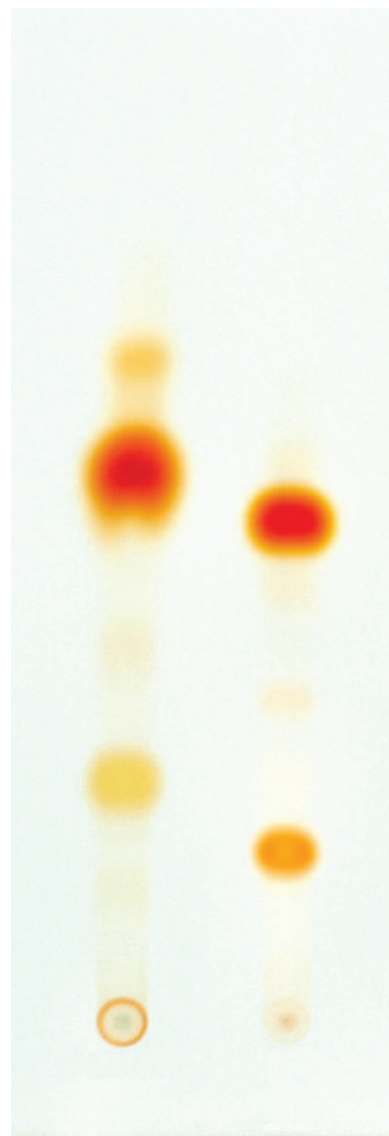


Fig. 4. The thin-layer chromatograms of 2 patch test preparations labelled Disperse Orange 3. The reference substance could be detected in the sample to the left but not in the sample to the right.

DB 106 in preparations labelled DB 124 1.0% varied from 0.06% to 0.005% when analyzed with HPLC, corresponding to 1/17 to 1/200 of stated DB 124 concentration. The only patch test preparation from supplier 3 contained the highest concentration of DB 106, also visualized as a more intense spot with R_f corresponding to DB 106, when analyzed on the TLC plate. 10/14 preparations showed a distinct pink spot (Fig. 3), but this spot was missing in the only preparation from supplier 3. Another 3 test preparations from supplier 1, with expiry date in 1995, 1999, and 2005, respectively, all showed a corresponding but more faint-coloured pink spot on the TLC plates, despite having ordinary concentrations when analyzed with HPLC.

Disperse Blue 35

We have not been able to identify any reference substance concerning DB 35. The 12 patch test preparations labelled DB 35 from various departments showed a similar pattern, lacking a main spot, but with at least 5 equivalent spots in the thin-layer chromatograms (Fig. 2) and with many peaks in the HPLC chromatograms.

Disperse Yellow 3

The average extraction recovery was 99.5% (CV 7.1%). The mean concentration of DY 3 in the 14 patch test preparations was 0.44% (0.27–0.61). The TLC plates of the DY 3 preparations showed 2 spots, 1 of them in a strong yellow colour (Fig. 2), with R_f corresponding to the R_f of DY 3. The concentration of DY 3 and the TLC pattern were independent of supplier and expiry date when comparing the various preparations.

Disperse Orange 1

The average extraction recovery was 95.4% (CV 5.1%). The mean concentration of DO 1 in the 12 patch test preparations was 0.40% (0.34–0.57). All but 1 preparation showed an equal TLC pattern, having 1 spot with R_f corresponding to the R_f of DO 1 (Fig. 2). No difference was seen because of the expiry date. The remaining preparation showed 2 extra faint-coloured spots. This preparation expired in April 2005.

Disperse Orange 3

The average extraction recovery was 96.1% (CV 9.3%). In 4 test preparations, of which 3 were produced by supplier 1 and 1 at the laboratory in Malmö, DO 3 could not be detected (detection limit 0.009%) when analyzed with HPLC (Table 1). The mean concentration of DO 3, excluding the 4 mentioned divergent test preparations, was 0.68% (0.25–0.93). 11/15 test preparations had comparable concentration of DO 3. The thin-layer chromatograms of these 11 preparations showed a similar pattern with 5 spots, the main spot included. The R_f of this spot corresponded to the R_f of DO 3. Another 4 preparations had a totally divergent pattern, with 4 other spots, another main spot, and lacking the spot corresponding to the main spot in the aforementioned 11 preparations (Fig. 4). 8 of these 11 preparations also had a bright yellow spot with a lower R_f , lacking in the thin-layer chromatograms of the preparations with a divergent main spot. The remaining 3 preparations showed weaker main spots and weaker bright yellow spots in the

thin-layer chromatograms. These preparations contained 0.25% to 0.38% of DO 3 when analyzed with HPLC. Of these 3 preparations, representing 3 suppliers, 1 preparation expired >10 years ago whereas the other had not expired at arrival. The main variation in TLC pattern between the test preparations labelled DO 3 is demonstrated in Fig. 4.

Disperse Red 1

The average extraction recovery was 92.0% (CV 21.7%). The mean concentration of DR 1 in the 14 patch test preparations was 0.49% (0.35–1.03). 2 preparations, both from supplier 2, contained 1.01% and 1.03%, respectively, whereas the remaining 12 preparations contained <0.50% of DR 1 (Table 1). All preparations had a main spot, with R_f corresponding to the R_f of DR 1 when visualized on TLC plates (Fig. 2). 10/14 preparations had 8 spots, 3 had 7 spots, missing a faint-coloured orange spot. 1 preparation from supplier 2 had an extra, light red, spot. This preparation also missed the faint-coloured orange spot. In the only preparation from supplier 3, several spots were missing and the main spot was slightly weaker than the average. No difference in TLC pattern was seen because of the expiry date. The preparations purchased from supplier 2 had a higher concentration of DR 1 compared with the average. This was also seen on the corresponding TLC plates as spots with a more intense colour.

Disperse Red 17

The average extraction recovery was 94.7% (CV 9.3%). The mean concentration of DR 17 in the 14 patch test preparations was 0.35% (0.27–0.50). All preparations had a red main spot, with R_f corresponding to the R_f of DR 17, and a weaker orange-coloured spot with a higher R_f (Fig. 2). 12 preparations showed a similar TLC pattern with 3 spots, 1 preparation had 1 extra faint-coloured spot, and the remaining preparation, representing supplier 2 and with expiry date in 1997, showed 2 extra spots.

Discussion

Information about textile dyes can be found in the CI (11). Each dye has a CI generic name composed of the application class, the colour, and a number, e.g. DO 1. Furthermore, the dyes with a defined structure have a CI structure number, e.g. DO 1 has the CI number 11080. CI also contains structure images, uses, commercial names, and producers of the dyes. CAS, a division of

the American Chemical Society (12), also assigns identifiers to chemicals that have been described in the literature by giving them CAS numbers, unique numerical identifiers for chemical compounds. DB 35 has a defined CAS number but no specified chemical formula, and CI number is therefore missing. The remaining 7 DDs investigated in our study have both CAS and CI numbers and are thus described with defined molecular structures (Fig. 1).

According to a study by Fousereau and Dallara in 1986, commercial textile dyes made by Chemotechnique Diagnostics containing DY 3, DO 1, and DO 3 seemed pure when analyzed with TLC whereas DB 35, DR 1, and DR 17 seemed impure (13). Dyes from various suppliers also differed, although their CI number was the same. Le Coz et al. reported in 2004 at the European Society of Contact Dermatitis meeting in Copenhagen that test preparations labelled DO 3 contained DO 31 instead when analyzed with TLC and HPLC (14). In a review from 2000, Hatch and Maibach encouraged researchers to verify the identity and purity of the dyes used for patch testing (1).

According to studies earlier performed at the Malmö department, the isocyanate patch test preparations used at various dermatology departments showed a 'huge' variation in the content of isocyanates (15, 16). The present investigation demonstrates that most of the DD patch test preparations contain 20–50% of the labelled concentration. The patch test preparations prepared at the Malmö department (department M in Table 1) showed the same low concentrations of the declared DDs, although they contained measured material that constituted 1.0% of the Pet. patch test preparation. This indicates that most of the raw materials used at the Malmö department contained 39–76% contaminants and/or other substances (Table 1). As estimated from the TLC and HPLC analyses, the contaminants that were coloured or visible in UV radiation could only constitute a minor part of this massive contamination. The results of the analyses (Table 1) indicate that the producers of patch test preparations have used raw materials of similar quality as the Malmö department with some exceptions mainly regarding patch test preparations of DO 3 and DR 1, for which some suppliers obviously have used raw materials of higher purity. According to online information given by an Indian manufacturer of textile dyes, commercial DDs can contain as high as 60% lignin sulfonate (synonym: lignosulfonate), a dispersing agent. This substance might be added as a functional additive as dispersing agents often are used in dye baths

when dyeing synthetic fibres with DDs (17). If lignin sulfonate is present in some DDs, it has probably little influence on the allergenic potency of these dyes. Lignin sulfonate has been patch tested at our department in more than 400 patients tested with no positive patch test reaction. In the literature, we have found only one report on contact allergy to calcium lignosulfonate (18).

Some of the DO 3 labelled preparations did not contain DO 3. TLC and HPLC analyses showed that these patch test preparations instead contained another orange dye. The TLC analysis shown in Fig. 4 clearly shows the difference between the 2 orange dyes.

We have asked both the producers of commercial DDs and of patch test preparations with DDs whether they take into account the purity of the substances when calculating the concentrations in the dyes and in the patch test preparations. 2 chemical companies have answered that they analyze each new lot with UV and infrared spectroscopy and with TLC. The results are compared with a 'working standard' but not to a 'highly purified dye standard'. None of the producers of patch test preparations has answered, despite repeated inquiries.

According to Isaksson et al. (19), producers of patch test preparations apply a permitted limit for the content of patch test preparations until the expiry date of $\pm 20\%$ of the concentration given on the label. However, 83/90 (92%) patch test preparations contained $< 80\%$ of the stated concentration. 59/90 (66%) of the preparations had passed the expiry date when analyzed, and the results did not indicate any correlation between the expiry date and declining concentration (Fisher's exact test, 2 sided).

This study indicates that all test preparations of DB 124 contain DB 106 and vice versa, with different content in preparations from various suppliers. A high degree of cross reactivity between these substances has been described earlier and been attributed to a close structural similarity (20, 21). The possible content of DB 106 in the test preparation labelled DB 124 and vice versa could be an explanation to some of the simultaneous allergic patch test reactions. Hydrolysis of the ester group in DB 124 will convert this substance into DB 106 and acetic acid, a metabolic reaction that can occur in the skin (22). The possibility of allergic test reactions to unidentified impurities or synergistic or additive effects between the substances makes the interpretation of the test results even more difficult.

In 4/15 test preparations labelled DO 3, this DD could not be detected, implying that 4 departments tested with the wrong substance. Especially

concerning DO 3, the use of test preparations containing divergent concentrations compared with the label, as well as the wrong substance in some samples, makes the comparison of the test results from various departments unreliable or impossible. The fact that all the test preparations have several peaks when analyzed with HPLC and various spots are seen on the TLC plates also raises the question whether the patients with positive test reactions to these test preparations react to the dye or to the impurities. Furthermore, although the coloured spots in the thin-layer chromatograms visualize impurities, there may be additional impurities not seen in visible light or in UV radiation but giving rise to contact allergy. The aforementioned extra spot seen on all the TLC plates when inspected in UV radiation was also seen on the plate made from pure Pet., indicating that this spot was not derived from the DDs. The nature and importance of this constituent in the test preparations has not yet been investigated.

Patch testing with impure test preparations, with preparations containing a lower and varying concentration compared with the label or with the wrong substance may have implications for the individual patient concerning difficulties in diagnosis and prevention according to the test results. It may also result in misleading or incorrect advices related to contact allergy to DDs with regard to the general preventive measures concerning contact allergy and allergic contact dermatitis from DDs.

CEN, the European Committee for Standardization, decided in 2003, based on a survey of significant contact sensitizers in the environment among metals, polymers, fragrances, preservatives, and DDs, that there was a great need to develop standards for analytical methods applicable to defined allergenic substances in materials and products. The DDs (DB 1, DB 106, DB 124, DB 35, DY 3, DY 9, DO 3, DR 1, and DR 17) belonged to the allergens given the highest priority for standardization. However, as the standardization work required a methodology for analysis of the defined DDs causing contact allergy, and it was known that the commercial dyes were not pure, the work was postponed (23). We hope that the results of our investigations will contribute to the knowledge of the properties of DD patch test preparations and emphasize the importance of performing comparable chemical analyses of the test preparations used in various departments to enable comparisons of patch test results. However, as we still remain uncertain if the sensitizer is the DD or a contaminant in the patch test preparations, further investigations and comparative patch testing with test preparations

containing DD reference substances as well as with the commercial DDs that are currently used for dyeing textiles are needed to define their capacity to give allergic reactions. Nevertheless, we hope that our present study will provide a basis for the future work on general prevention concerning contact allergy and allergic contact dermatitis from DDs.

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