

Investigation of the sensitising potential of textile dyes using a biphasic protocol of the local lymph node assay

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INTRODUCTION

Disperse dyes are the main causative agents for textile dermatitis. Due to their lipophilic feature and molecular size these dyes have easy skin penetration, which is a requirement for inducing allergic contact dermatitis. A few reports of dermatitis due to other dyes can be found in the literature.

Reports of dye-related contact sensitization include exposure to dyes in clothing, cosmetics, spectacle frames, felt-tip marker pens, shoes and a variety of other consumer products.

The aim of the present study was to investigate the sensitising potential of various disperse dyes using a modified protocol of the local lymph node assay.

MATERIAL & METHODS

Chemicals

Disperse blue 1 (CAS no. 2475-45-8), Disperse blue 35 (CAS no. 12222-75-2), Disperse blue 106 (CAS no. 68516-81-4), Disperse blue 124 (CAS no. 61951-51-7), Disperse yellow 3 (CAS no. 2832-40-8), Disperse orange 3 (CAS no. 730-40-5), Disperse orange 37 (CAS no. 13301-61-6), Disperse red 1 (CAS no. 2872-52-8) were purchased from Sigma-Aldrich Chemie GmbH, Steinheim, Germany. Solutions were prepared freshly for each application in dimethyl sulfoxide (DMSO; Merck KGaA, Darmstadt, Germany).

Treatment of animals

Female Balb/c mice (age: 7 weeks, weight: 18-22 g) were treated using a "biphasic protocol" (Fig. 1).

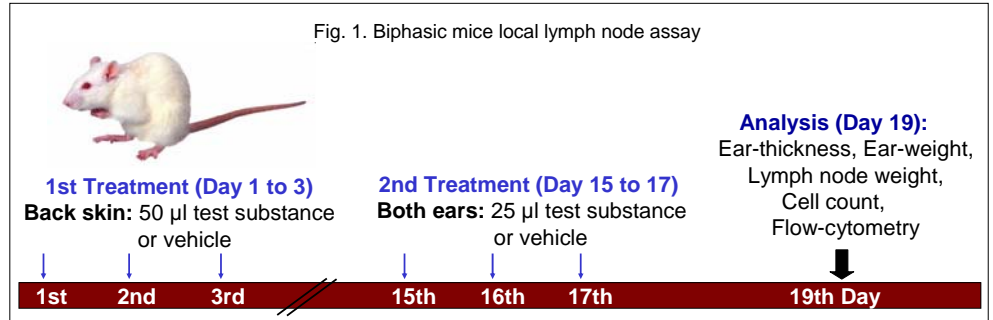
On days 1-3, the animals were shaved over a surface of approximately 2 cm² on their back. This area was treated once daily on days 1-3 with 50 µl of the test solution in DMSO, with the below mentioned concentrations. All the mice remained untreated on days 4-14. The control animals were treated with the vehicle alone.

On days 15-17, the treatment was repeated once a day for 3 consecutive days on the dorsum of both ears (25 µl per ear).

The animals were euthanized with deep CO₂ anaesthesia on day 19, and the lymph nodes were prepared.

Analysis: Following endpoints were analysed on day 19:

- Ear thickness (mm)
- Ear-punch weight (mg)
- Lymph node weight (mg)
- Lymph node cell count (million)
- FACS (% of CD4+, CD8+, CD19+, CD45+, CD45+/1A+, CD69+/CD4+ cells)



RESULTS

Out of the disperse dyes studied, Disperse blue 124 and Disperse blue 106 showed significant modulation in cell count (Fig.2), lymph node weight (Fig.3), ear-thickness (Fig.4), ear-punch weight (Fig.5) and cell-surface markers (Table 1), as compared to other dyes.

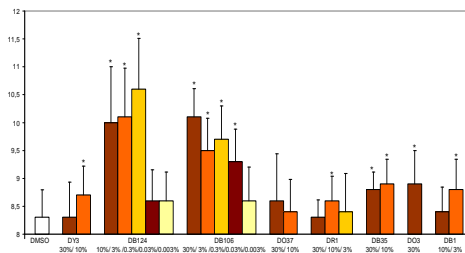


Fig 2. Cell count (Mean ± SD) values from lymph node of mice. *indicates significant difference at p<0.05 (t-test) between vehicle control and treated animals

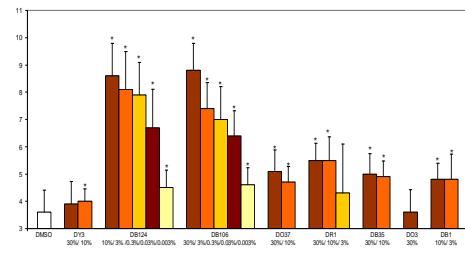


Fig 3. Lymph node weight (Mean ± SD) values. *indicates significant difference at p<0.05 (t-test) between vehicle control and treated animals

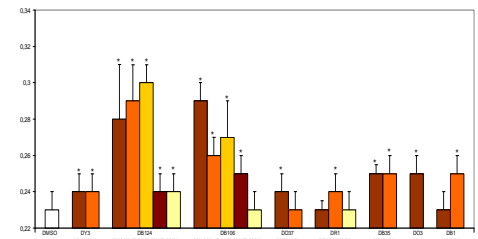


Fig 4. Ear-thickness (Mean ± SD) values. *indicates significant difference at p<0.05 (t-test) between vehicle control and treated animals

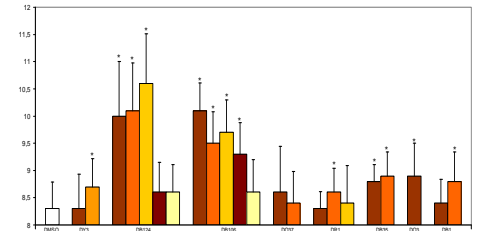


Fig 5. Ear-punch weight (Mean ± SD) values. *indicates significant difference at p<0.05 (t-test) between vehicle control and treated animals

Table 1. FACS end-points (Mean±SD) following exposure of mice to textile dyes in modified biphasic local lymph node assay

	CD4+	CD8+	CD45+	CD19+	CD45+/1A+	CD69+/CD4+
Control	47.7±4.3	20.8±2.1	31.4±4.6	20.1±2.5	21.4±3.7	10.4±1.3
DY3 30%	43.8±5.6	21.2±3.1	34.3±1.4	26.7±2.2*	31.7±4.3*	12.9±0.67*
DY3 10%	43.5±2.0*	21.0±2.6	32.2±0.98	31.4±4.5*	28.8±6.4*	10.1±1.0
DB124 10%	40.7±4.1*	18.3±1.7*	36.3±3.1*	24.4±3.9*	31.0±2.9*	16.2±4.4*
DB124 3%	37.4±5.5*	15.6±3.4*	40.0±1.3*	41.0±5.0*	42.3±8.7*	13.3±2.0*
DB124 0.3%	39.6±2.4*	18.7±2.0*	34.4±3.5	34.8±2.0*	30.5±3.0*	13.7±1.6*
DB124 0.03%	38.8±3.8*	15.4±2.0*	38.5±4.5*	39.7±5.1*	32.1±5.0*	9.9±0.52
DB124 0.003%	40.6±1.5*	15.5±1.1*	32.3±2.3	35.8±2.4*	30.6±1.6*	10.2±1.2*
DB106 30%	39.6±3.3*	17.4±2.4*	36.7±3.6*	26.8±3.5*	32.5±5.1*	14.6±2.3*
DB106 3%	38.4±3.5*	16.6±3.6*	40.4±2.5*	39.0±3.0*	40.7±8.7*	14.0±1.5*
DB106 0.3%	38.5±3.7*	19.0±2.2*	34.0±4.4	36.4±5.0*	31.1±4.1*	12.1±1.1*
DB106 0.03%	36.0±1.6*	16.3±1.6*	38.3±3.4*	41.1±2.6*	34.9±2.8*	9.8±0.78
DB106 0.003%	40.4±1.2*	15.1±1.3*	31.3±2.0	27.1±3.7*	31.3±1.6*	9.9±0.82
DO37 30%	32.0±4.1*	15.4±2.3*	34.0±2.5	32.0±4.1*	37.5±3.1*	10.2±1.8
DO37 10%	41.7±4.1*	21.0±2.2*	33.7±4.1	28.1±3.6*	30.7±6.0*	9.6±1.2
DR1 30%	36.1±5.2*	16.0±1.7*	32.4±1.5	33.7±3.3*	38.6±5.3*	9.4±1.5
DR1 10%	43.4±2.5*	21.6±1.7*	33.8±3.7	26.6±4.2*	27.6±3.0*	10.7±1.8
DR1 3%	46.1±3.3	20.2±1.5	32.1±2.9	25.9±3.0*	24.0±3.1*	9.6±0.50
DB35 30%	36.3±4.7*	16.7±2.0*	35.1±2.0*	39.8±6.1*	45.1±5.4*	9.9±1.9
DB35 10%	40.0±2.1*	19.3±1.8*	38.1±3.5*	34.5±2.0*	32.1±1.8*	10.1±1.3
DO3 30%	44.0±3.3*	19.1±1.4*	31.0±2.1	27.1±3.7*	29.3±4.1*	10.5±1.3
DB1 10%	40.2±3.0*	17.1±1.7*	36.1±2.8*	33.2±7.9*	35.9±8.2*	10.9±1.4
DB1 3%	40.0±2.1*	17.4±2.0*	44.1±2.6*	38.7±2.3*	36.0±2.4*	10.0±1.4

CONCLUSION

Our results show that the biphasic protocol is suitable in studying the sensitising potential of textile dyes. The disperse dyes investigated in our study could be arranged on the basis of their sensitising potency in the following order: DB124 > DB106 > DR1 > DB1 > DO37 > DB35 > DY3 > DO3