

# Sensitizing capacity of Disperse Orange 1 and its potential metabolites from azo reduction and their cross-reactivity pattern

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## Summary

**Background.** Simultaneous contact allergies to Disperse Orange 1, 4-nitroaniline and *p*-aminodiphenylamine (PADPA), as well as to other disperse azo dyes and to *p*-phenylenediamine (PPD), have been reported. Cross-reactivity is one of the possible explanations for simultaneous reactions between PPD and disperse azo dyes. Some metabolites from the azo reduction of these disperse azo dyes could be sensitizers, as human skin bacteria produce azo reductases.

**Objectives.** To investigate the sensitizing capacity of Disperse Orange 1, PADPA and 4-nitroaniline, and the cross-reactivity between these substances and Disperse Yellow 3, its potential metabolites from azo reduction (4-aminoacetanilide and 2-amino-*p*-cresol), and PPD.

**Method.** The guinea-pig maximization test was used.

**Results.** It was found that both Disperse Orange 1 and PADPA are strong sensitizers and cross-react with each other. We were unable to sensitize guinea-pigs with 4-nitroaniline tested in equimolar concentrations to Disperse Orange 1.

**Conclusions.** The results indicate that patients sensitized primarily to Disperse Orange 1 will also react to PADPA, which can be found mainly in hair dyes. PPD, 4-nitroaniline, 4-aminoacetanilide, 2-amino-*p*-cresol and Disperse Yellow 3 did not show any cross-reactivity with Disperse Orange 1 or PADPA.

**Key words:** azo dyes; cross-reactivity; Disperse Orange 1; metabolites; *p*-aminodiphenylamine; *p*-phenylenediamine.

Disperse Orange 1 is a textile azo dye. It is known to be a sensitizer in humans (1), but this has never been investigated in animal studies. Simultaneous contact allergies to Disperse Orange 1, 4-nitroaniline, and *p*-aminodiphenylamine (PADPA), as well as to other disperse azo dyes and to *p*-phenylenediamine (PPD), have

been reported (2–4). Cross-reactivity is one of the possible explanations for simultaneous reactions between disperse azo dyes, PPD, and its derivatives. Some metabolites from the azo reduction of the disperse azo dyes may be the primary sensitizers in cases of contact allergy to these dyes, as it has been shown that human skin bacteria produce azo reductases and that azo reduction takes place in the human skin (5, 6). Another explanation may be common contaminants. In humans, however, it is impossible to show whether the positive patch test reactions are manifestations of cross-reactivity or result from concomitant sensitization to these chemicals. The guinea-pig maximization test (GPMT) is a useful tool for the investigation of the sensitizing capacity of a

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chemical and for the elucidation of cross-reaction patterns among structurally related sensitizers (7).

In order to investigate the sensitizing capacity of Disperse Orange 1 and its two metabolites from azo reduction (PADPA and 4-nitroaniline), and the cross-reactivity to Disperse Yellow 3, its potential metabolites from azo reduction (4-aminoacetanilide and 2-amino-*p*-cresol), and PPD, we conducted this study with the GPMT.

## Materials and Methods

### Substances

Acetone of analytical grade was obtained from Scharlau Chemie S. A. (La Jota, Barcelona, Spain). Disperse Orange 1 and Disperse Yellow 3 had been purified and identified earlier at the Malmö department from commercial Disperse Orange 1 and Disperse Yellow 3 (8), purchased from Chemotechnique Diagnostics (Vellinge, Sweden).

PPD was bought from Chemotechnique Diagnostics. 4-Nitroaniline, PADPA, 4-aminoacetanilide and 2-amino-*p*-cresol were bought from Sigma Aldrich (Steinheim, Germany) (Fig. 1). The general and specific purities of the substances are given in Table 1 (9).

Freund's complete adjuvant (FCA) was obtained from Pierce (Rockford, IL, USA). 2-Methylol phenol was bought from Fluka Chemie AG (Buchs, Switzerland). Propylene glycol was obtained from VWR International S.A.S. (Fontenay-sous-Bois, France), sodium lauryl sulfate from Acros Organics (Geel, Belgium), dimethylacetamide from Sigma Chemical Co. (St Louis, MO, USA), and ethanol from Kemetyl AB (Haninge, Sweden).

### Gas chromatography–mass spectrometry (GCMS)

Chemical characterization of the substances used in the GPMT was performed by GCMS with an Agilent 6890N gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled with a Jeol GCmate II mass spectrometer (Jeol Datum Ltd, Tokyo, Japan). Chromatographic separation was performed with a 30-m-long capillary column (0.25 µm film thickness of methyl silicone, internal diameter 0.250 mm; Agilent Technologies). Helium was used as the carrier gas, with a flow rate of 1 ml/min. One microlitre of the sample was injected into a splitless inlet heated at 250 °C. The GC column temperature programme was: 3 min at 70 °C, then 8 °C/min to 300 °C, and finally 10 min at 300 °C. The GCMS interface was maintained at 250 °C. Electron-impact spectra were recorded for *m/z* 50–600, with a scan duration of 0.3 seconds and an interscan delay of 0.2 seconds, an

ion source at 250 °C, and 70 eV. The National Institute of Standards and Technology (Gaithersburg, MD, USA) library of mass spectra was used for identification.

### Direct inlet mass spectrometry

The Jeol GCmate II mass spectrometer was used with the gas chromatograph disconnected but with the same settings of the MS parameters. Approximately 1 µg of the substance to be analysed was introduced into the MS, and gradually heated from room temperature to 400 °C over a period of 25 min.

### Ethics

The study was approved by the Lund Ethical Committee on Animal Experiments, Lund, Sweden, and conducted in accordance with ethical standards (approval No. M 28–12).

### Guinea-pig maximization test

The GPMT was performed according to the original description (10). In order to standardize the test and objectify the evaluation of the patch test reactions, some modifications were made, including statistical calculations, blind reading, and a positive control group (9, 11, 12). Female albino guinea-pigs weighing 400 g ( $\pm$  20 g) of the Hartley–Dunkin strain (HP Lidköpings Kaninfarm, Lidköping, Sweden) were used.

*Topical irritancy.* Topical irritancy was determined by applying different concentrations of each substance used for induction as a closed patch test for 2 days on both the neck and the flank of three or four animals. One week before testing, the animals were pretreated with FCA. Concentrations that did not cause irritation and did not dye the skin of the animal were chosen for topical induction and elicitation.

*Concentrations.* Equimolar concentrations were used for all of the substances used in the study. The concentrations used for induction and challenge are given in Table 2.

*Induction.* Twenty-four test animals were used for induction, according to the following procedure:

DO: three intradermal injections in a row at the site of each shoulder were given:

- (1) 0.1 ml of FCA in water 40% wt/vol (FCA/water 50:50 vol/vol);

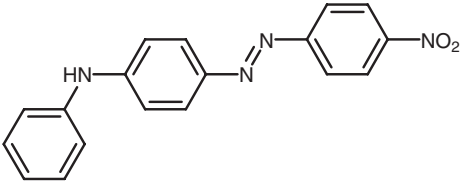
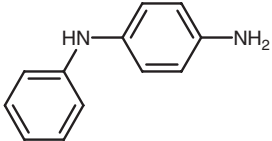
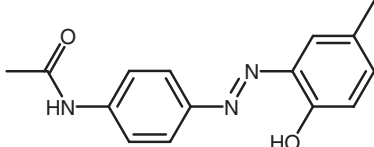
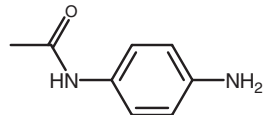
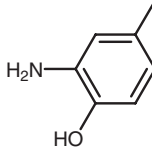
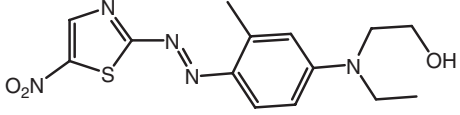
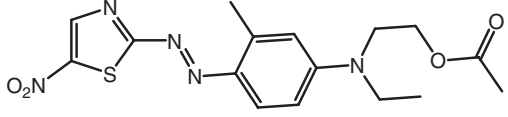
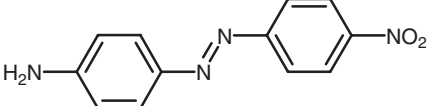
|                                                                                     |                                                                                          |
|-------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
|    | <b>Disperse Orange 1</b><br>CAS: 2581-69-3; C.I.: 11080<br>MW: 318; log $P_{o/w}$ : 5    |
|    | <b>p-aminodiphenylamine</b><br>CAS: 101-54-2<br>MW: 184; log $P_{o/w}$ : 2.7             |
|    | <b>4-nitroaniline</b><br>CAS: 100-01-6<br>MW: 138; log $P_{o/w}$ : 1.01;                 |
|    | <b>Disperse Yellow 3</b><br>CAS: 2832-40-8; C.I.: 11855<br>MW: 269; log $P_{o/w}$ : 3.48 |
|   | <b>4-aminoacetanilide</b><br>CAS: 122-80-5<br>MW: 150; log $P_{o/w}$ : 0.14              |
|  | <b>2-amino-p-cresol</b><br>CAS: 95-84-1<br>MW: 123; log $P_{o/w}$ : 1.33                 |
|  | <b>p-phenylenediamine</b><br>CAS: 106-50-3<br>MW: 108; log $P_{o/w}$ : 0.43              |
|  | <b>Disperse Blue 106</b><br>CAS: 68516-81-4; CI: 111935<br>MW: 335; log $P_{o/w}$ : 2.9  |
|  | <b>Disperse Blue 124</b><br>CAS: 15141-18-1; CI: 111938<br>MW: 377; log $P_{o/w}$ : 3.8  |
|  | <b>Disperse Orange 3</b><br>CAS: 730-40-5; CI: 11005<br>MW: 242; log $P_{o/w}$ : 2.6     |

Fig. 1. Chemical structure, CAS numbers, Colour Index (C.I.) numbers and molecular weights (MW) for the investigated substances.

**Table 1.** General and specific purity of the substances used in the guinea-pig maximization test

| Substances, obtained from the manufacturers | Concentration indicated on the label | Results from GCMS and DIMS |                |                              |                   |                           |                    |                            |
|---------------------------------------------|--------------------------------------|----------------------------|----------------|------------------------------|-------------------|---------------------------|--------------------|----------------------------|
|                                             |                                      | Used substances            |                |                              |                   |                           |                    |                            |
|                                             |                                      | Disperse Orange 1          | 4-Nitroaniline | <i>p</i> -Aminodiphenylamine | Disperse Yellow 3 | 2-Amino- <i>p</i> -cresol | 4-Aminoacetanilide | <i>p</i> -Phenylenediamine |
| Disperse Orange 1                           | Not stated                           | > 99%*                     | ND             | ND                           | ND                | ND                        | ND                 | ND                         |
| 4-Nitroaniline                              | ≥ 99%                                | ND                         | > 99%          | ND                           | ND                | ND                        | ND                 | ND                         |
| <i>p</i> -Aminodiphenylamine                | 98%                                  | ND                         | ND             | 98%                          | ND                | ND                        | ND                 | ND                         |
| Disperse Yellow 3                           | Not stated                           | ND                         | ND             | ND                           | > 99%†            | ND                        | ND                 | ND                         |
| 2-Amino- <i>p</i> -cresol                   | 97%                                  | ND                         | ND             | ND                           | ND                | 99%                       | ND                 | ND                         |
| 4-Aminoacetanilide                          | 99%                                  | ND                         | ND             | ND                           | ND                | ND                        | 99%                | ND                         |
| <i>p</i> -Phenylenediamine                  | Not stated                           | ND                         | ND             | ND                           | ND                | ND                        | ND                 | 99%                        |

DIMS, direct injection mass spectroscopy; GCMS, gas chromatography–mass spectroscopy; ND, not detected (detection limit <0.1%).

Purification of Disperse Orange 1 and Disperse Yellow 3 was performed at the Department of Occupational and Environmental Dermatology, Malmö, Sweden.

\*Concentration before purification 15.2%.

†Concentration before purification 40.6%.

**Table 2.** Concentrations (% wt/vol) used for induction and challenge in the guinea-pig maximization test

| Substance                    | Intradermal sensitization | Topical sensitization | Challenge I | Challenge II |
|------------------------------|---------------------------|-----------------------|-------------|--------------|
| Disperse Orange 1            | 1.20                      | 2.30                  | 0.57        | 0.57         |
| 4-Nitroaniline               | 0.50                      | 1.0                   | 0.25        | 0.25         |
| <i>p</i> -Aminodiphenylamine | 0.65                      | 1.30                  | 0.33        | 0.33         |
| Disperse Yellow 3            | –                         | –                     | –           | 0.49         |
| 2-Amino- <i>p</i> -cresol    | –                         | –                     | –           | 0.22         |
| 4-Aminoacetanilide           | –                         | –                     | –           | 0.27         |
| <i>p</i> -Phenylenediamine   | –                         | –                     | –           | 0.20         |

- (2) 0.1 ml of a solution of induction substance (wt/vol) dissolved in acetone and diluted in propylene glycol;

- (3) 0.1 ml of a mixture of 40% wt/vol FCA in acetone/propylene glycol and with the same concentration of induction substance as in (2).

D6: pretreatment of a 2 × 4-cm area on the neck for topical induction with 0.2 ml of sodium lauryl sulfate 10% wt/vol in dimethylacetamide/acetone/ethanol 99.5% 4:3:3 vol/vol/vol 1 day before topical application of the induction substance.

D7: topical induction on the neck with 0.2 ml of induction substance on a 2 × 4-cm piece of filter paper (130 g/m<sup>3</sup>; Munktell1 Filter AB, Grycksbo, Sweden) placed on Durapore (3M Health Care, St Paul, MN, USA). The patches were covered with impermeable plastic adhesive tape (Acrylastic; Beiersdorf AG, Hamburg, Germany) and held in place with an adhesive bandage. The patch was left on for 2 days.

**Controls.** Twelve controls were given exactly the same treatment as described for the test animals, but with the

induction substance excluded. In addition, six controls were given the known sensitizer 2-methylol phenol. These animals were used as positive controls in order to objectify the evaluation of test reactions and as an indication of the accuracy of the induction procedure (9).

### Challenge

**D21, Challenge I, sensitization rate (right flank, two patches):** 12 of 24 test animals were challenged with the induction substance on both the cranial and the caudal patch, and 6 + 6 test animals were challenged with the induction substance on either the cranial patch or the caudal patch, with vehicle alone on the other. AI-test® (Imeco AB, Södertälje, Sweden) on Durapore was used for patch testing. Thirty microlitres of the induction substance used for induction diluted in acetone was applied. Acrylastic and an outer layer of Durapore held the tests in place. The patches were removed after 1 day. Six of 12 control animals were tested with the induction substance on both patches, and 3 + 3 control animals were tested with the induction substance on either the cranial or the caudal patch, with vehicle alone on the other patch (13).

**Challenge II, cross-reactions (left flank, six patches): on the same occasion as challenge I:** The same 24 test animals as in challenge I and 12 control animals were, in addition to 4-nitroaniline and PADPA, challenged with PPD, Disperse Yellow 3, 4-aminoacetanilide, and 2-amino-*p*-cresol. The positions of test substances were based on a Latin square table.

**Evaluation. D23:** The minimum criterion for an allergic (positive) reaction is a confluent erythema. All tests were evaluated blindly 1 day after the patches had been removed, that is, 2 days after the application. First, the right flanks (challenge I) were read, and thereafter, still blindly and without knowledge of the readings of the right flanks, the left flanks (challenge II) were read.

The procedure concerning the control group sensitized and challenged with 2-methylol phenol is described elsewhere (9).

Induction for each of the three substances was performed on different occasions, and freshly made solutions of the substances were always used.

#### Statistical calculation

The number of positive animals within the test group was compared with the number of positive animals in the control group. The number of positive test animals was also compared with the number of positive animals tested with vehicle only. Among the animals challenged with the induction substance on both the cranial and caudal patches (12 test animals and 6 control animals), only one of the patches chosen in advance was included. Statistical significance was calculated with a one-sided Fisher's exact test (comparing control and test animals) and with the McNemar test (comparing test substance with the vehicle in the same animal). When a significant value ( $p < 0.05$ ) was obtained both in the comparison between the test group and the controls tested with the allergen and the comparison between positively tested animals and animals tested with the vehicle alone, the compound was considered to be a sensitizer.

## Results

As induction for each substance was performed on different occasions, the results represent data from three different experiments.

#### Purity

The investigation of the purity of the test substances showed that the general purity was  $\geq 99\%$ , with the

exception of PADPA, for which the general purity was 98%. The specific purity of all used substances was high (Table 1).

#### Sensitizing capacity

Disperse Orange 1 and PADPA were found to be strong sensitizers in the guinea-pig. Positive reactions were seen to both Disperse Orange 1 and PADPA in 22 of 24 animals. Two control animals had reactions to Disperse Orange 1, and two control animals reacted to PADPA ( $p < 0.001$ ). Only 5 of 24 animals had positive reactions to 4-nitroaniline, and 2 control animals also reacted to this substance ( $p > 0.3$ ) (Table 3).

#### Cross-reactivity

The results of the tests for cross-reactivity are given in Table 4. PADPA gave a positive test reaction in 21 of 24 animals sensitized to Disperse Orange 1 ( $p < 0.001$ ) (Table 4). Two animals that were negative for PADPA were positive for Disperse Orange 1. One was negative both for PADPA and for Disperse Orange 1.

Disperse Orange 1 was positive in 23 of 24 animals sensitized to PADPA and in 6 of 12 controls ( $p < 0.001$ ). Although PPD was positive in 17 of 24 animals, it was also positive in 7 of 12 controls ( $p > 0.3$ ).

Cross-reactivity in the animal group in which 4-nitroaniline was the induction substance was not assessed, because sensitization to this substance failed.

## Discussion

#### Purity

When assessing contact sensitization and cross-reactivity, it is very important to ensure that experiments are performed with as pure substances as possible. It could be that contaminants or impurities are allergens by themselves,

**Table 3.** Sensitizing capacity of Disperse Orange 1, *p*-aminodiphenylamine, and 4-nitroaniline

| Induction substance          | T/n   | C/n  | V/n  | P/n |
|------------------------------|-------|------|------|-----|
| Disperse Orange 1            | 22/24 | 2/12 | 0/12 | 1/6 |
| <i>p</i> -Aminodiphenylamine | 22/24 | 2/12 | 0/12 | 1/6 |
| 4-Nitroaniline               | 5/24  | 2/12 | 2/12 | 4/6 |

C, number of positive test reactions to the induction substance in control animals; n, number of tested animals in the four groups T, C, V, and P; P, number of positive test reactions to 2-methylol phenol in the positive control group; T, number of positive test reactions to the induction substance in test animals; V, number of positive test reactions to the vehicle in test animals.

**Table 4.** Cross-reactions between Disperse Orange 1 (DO1), Disperse Yellow 3 (DY3), their potential metabolites and *p*-phenylenediamine (PPD) in 24 test and 12 control animals

| Induction substance | Challenge substances |          |                |          |       |          |     |          |       |          |       |          |     |          |
|---------------------|----------------------|----------|----------------|----------|-------|----------|-----|----------|-------|----------|-------|----------|-----|----------|
|                     | DO1                  |          | 4-Nitroaniline |          | PADPA |          | DY3 |          | 2-APC |          | 4-AAA |          | PPD |          |
|                     | +                    | <i>p</i> | +              | <i>p</i> | +     | <i>p</i> | +   | <i>p</i> | +     | <i>p</i> | +     | <i>p</i> | +   | <i>p</i> |
| DO1                 |                      |          |                |          |       |          |     |          |       |          |       |          |     |          |
| Test                | 22                   | < 0.001  | 1              | > 0.3    | 21    | < 0.001  | 0   | > 0.3    | 1     | > 0.3    | 1     | > 0.3    | 1   | > 0.3    |
| Control             | 2                    |          | 0              |          | 0     |          | 0   |          | 0     |          | 0     |          | 1   |          |
| PADPA               |                      |          |                |          |       |          |     |          |       |          |       |          |     |          |
| Test                | 23                   | < 0.0028 | 1              | > 0.3    | 22    | < 0.001  | 2   | > 0.3    | 3     | > 0.3    | 1     | > 0.3    | 17  | > 0.3    |
| Control             | 6                    |          | 0              |          | 2     |          | 1   |          | 1     |          | 0     |          | 7   |          |
| 4-Nitroaniline      |                      |          |                |          |       |          |     |          |       |          |       |          |     |          |
| Test                | NA                   |          | 5              | > 0.3    | NA    |          | NA  |          | NA    |          | NA    |          | NA  |          |
| Control             |                      |          | 2              |          |       |          |     |          |       |          |       |          |     |          |

2-APC, 2-amino-*p*-cresol; 4-AAA, 4-aminoacetanilide; PADPA, *p*-aminodiphenylamine; +, number of positive guinea-pigs; NA, not assessed.

as was shown when patients were tested with the commercial Disperse Blue 106 and 124, Disperse Orange 1, and Disperse Yellow 3 (14, 15). The concept of general purity includes chemically undefined impurities that can have unknown biological significance (9). Investigation of the specific purity means detecting the presence of certain substances (e.g. degradation products or raw materials used for the synthesis) that may be expected and that might also have an impact on the results of a sensitization study (9). Ryberg et al. showed that Disperse Blue 124 was present in Disperse Blue 106 and vice versa in commercial patch tests preparations that were made from commercial dyes obtained from various manufacturers (8).

It is not so common to report on the purity of the substances used for animal studies (e.g. the local lymph node assay or the Beuhler test), and the possible influence of other chemicals present in the substance of interest remains. The substances used in this GPMT were confirmed to be of high general and specific purity, so the possibility that components other than the investigated substances influenced the results is almost completely excluded.

### Sensitization

Disperse dyes are the most common sensitizers among textile dyes (16), but few investigations have been performed on their sensitizing capacity.

Although the sensitizing capacity of a chemical can be determined with animal tests, human tests and *in vitro* assays, the GPMT is a standard method for analysing sensitization capacity and assessing cross-reactivity patterns at challenge (7, 9, 16).

The most investigated disperse dyes regarding their sensitizing capacity are Disperse Blue 124 and 106,

Disperse Orange 3, and Disperse Yellow 3 (17, 18). When discussing sensitizing capacity, it is important to know that the investigated substances do not contain other substances, but, in these aforementioned studies, the purity of the tested dyes was not reported. It is known from chemical investigations of commercial disperse dye patch test preparations that the difference between the concentration stated by the manufacturer and the detectable dye amount can differ up to five-fold (8).

Disperse Blue 106 has proven to be a strong contact allergen in the guinea-pig tests (19). It has been reported that the sensitization capacity of Disperse Blue 106 is similar to that of 2,4-dinitrochlorobenzene, which is one of the strongest contact allergens known (20, 21).

On the basis of the results from the biphasic murine local lymph node assay, Ahuja et al. grouped the disperse dyes according to their sensitizing potency (22). According to them, Disperse Yellow 3 and Disperse Orange 3 were weak sensitizers. Disperse Yellow 3 was found to be a weak sensitizer also in the GPMT and a modified local lymph node assay (17, 19). Interestingly, Disperse Orange 3 and Disperse Yellow 3 are two of the most frequently reported allergenic disperse dyes in humans, as shown by patch testing, probably because of a high level of exposure (23).

Sonnenburg et al. examined several disperse dyes and products from azo cleavage of these dyes in the loose-fit co-culture-based sensitization assay of primary human keratinocytes and of allogenic dendritic cell-related cells (24). In this assay, 4-nitroaniline and 4-aminoacetanilide showed no sensitizing potential, whereas Disperse Yellow 3 and 2-amino-*p*-cresol were categorized as extreme sensitizers. PADPA was found to be a sensitizer in the GPMT (25), but, to our knowledge, a GPMT has

not been performed previously with Disperse Orange 1. We sensitized 22 of 24 (92%) guinea-pigs with both substances. When the significance levels  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$  are used to designate sensitizers as weak, moderate or strong, respectively, Disperse Orange 1 and PADPA can be classified as strong sensitizers (9). They can be compared with strong sensitizers such as diglycidyl ether of Bisphenol F, phenyl glycidyl ether, or the main allergens in phenol-formaldehyde resins, for which GPMTs have been performed according to the same methodology (9, 12, 26, 27).

In our study, 4-nitroaniline at an equimolar concentration to Disperse Orange 1 did not show sensitizing capacity. This finding confirms results from other studies (24, 28), where 4-nitroaniline was found to be a non-sensitizer even when a higher concentration was used for sensitization in the GPMT (28).

### Cross-reactivity

It is only possible to study the cross-reaction patterns of suspected contact allergens when the exposure to them is controlled, as in the GPMT (29, 30).

PPD was reported to be a screening substance for textile dye-related dermatitis, but several clinical studies concluded that there was no statistical correlation between the positive patch test reactions to disperse dyes and PPD (31, 32), with the exception of Disperse Orange 3. In a few studies, a statistically highly significant association between contact allergies to PPD and Disperse Orange 3 was found (3, 4). Moreover, simultaneous reactions between Disperse Blue 124 and 106 are frequently observed (33). This is referred to as cross-reactivity in some publications, but it is now known that each dye may contain a low amount of the other dye. Another explanation is that Disperse Blue 124 can easily be converted to Disperse Blue 106 through hydrolysis in the skin (8).

In the present study, we showed that Disperse Orange 1 is a sensitizer in the GPMT. Whether the reactions to Disperse Orange 1 are reactions to this substance as such or to its metabolite PADPA cannot be stated. If Disperse Orange 1 is fully metabolized to PADPA during azo reduction on the skin by skin bacteria and/or in the skin, then there is not true cross-reactivity between these substances. Also, it has not been shown that Disperse Orange 1 is azo reduced *in vivo*. If only some or no Disperse Orange 1 is metabolized, then it is possible that true cross-reactivity with PADPA occurs. The same applies to the concomitant reactions to Disperse Orange 1 that were observed when guinea-pigs were induced with PADPA. The purity of Disperse Orange 1 was > 99%, and PADPA

was not detected in it, and PADPA did not contain Disperse Orange 1, so the presence of PADPA in Disperse Orange 1 or vice versa could not explain the observed challenge reactions.

To elucidate whether Disperse Orange 1 or PADPA is the primary sensitizer, testing of these two substances at equimolar concentrations and serial dilutions, both at induction and at challenge, would have been needed in a GPMT. Moreover, 4-nitroaniline is excluded from being the major sensitizer in cases of contact allergy to Disperse Orange 1, as it was positive in only one guinea-pig with a positive reaction to Disperse Orange 1. Also, this substance showed no sensitizing capacity when tested at an equimolar concentration to Disperse Orange 1.

Our results indicate that a person sensitized to Disperse Orange 1 will react to PADPA, but not to PPD. Humans can be exposed to PADPA from oxidative hair dyes or rubber items (34). PADPA can also be used in a textile dye synthesis, so it might remain in the final product and be transferred to textiles when they are dyed (34). Primary sensitization to PADPA causes contact allergy to Disperse Orange 1, as indicated by our study.

This study also shows that cross-reactivity among disperse azo dyes is not universal. Guinea-pigs sensitized to Disperse Orange 1 did not react to Disperse Yellow 3 when they were tested at equimolar concentrations. Some clinical reports have also shown that clinically relevant sensitization to only one disperse dye exists (4, 35, 36).

No cross-reactions were shown between PPD and Disperse Orange 1 as the sensitizer in our study. We noticed in our previous study that, when we patch tested patients sensitized to Disperse Orange 1 and not to the other disperse azo dyes, there were no concomitant positive patch test reactions to PPD (2). Interestingly, guinea-pigs induced with PADPA did not react at a statistically significant level when tested with PPD. Disperse Orange 1 and PADPA are less similar to one another than PADPA and PPD are to one another, so the lack of cross-reactivity between PADPA and PPD would suggest that cross-reactivity between Disperse Orange 1 and PADPA is unlikely. More experiments are needed to determine whether guinea-pigs sensitized to PPD would react when challenged with PADPA.

It is worth mentioning that reading of the patch test reactions to the coloured substances in guinea-pigs might be complicated, although, during irritancy testing, we chose concentrations that did not dye the patch test area (Fig. 2). As the epidermis of the guinea-pig contains fewer layers than that of

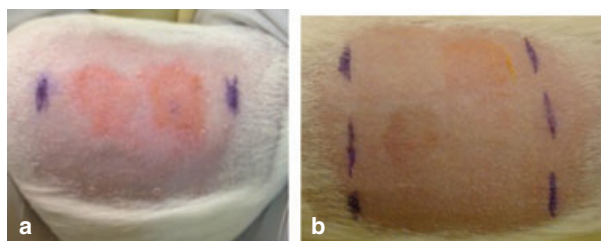


**Fig. 2.** Positive reactions in the guinea-pig when testing with coloured substances. (a) A clearly positive patch test reaction. (b) A coloured patch test area where erythema can be masked by the colour of the substance.

humans, a positive reaction to the sensitizer is mostly based on the erythema appearance. Blind readings and inclusion of the positive control group help to reduce possible bias of the over-interpretation of the positive or negative results.

Whether primary sensitization to PPD can cause cross-reactions to Disperse Orange 1 is not known. The GPMT study performed by Yamano et al. (25) showed that, when the guinea-pigs were sensitized with PPD, they reacted on challenge to PADPA, but when they were induced with PADPA, they did not react to PPD, even when challenged with a higher concentration, which was not equimolar to PADPA (25). It is possible that metabolic activation plays an important role in the sensitization capacity,

and differences in skin metabolism between animals and humans should also be taken into account.

## Conclusions

Disperse Orange 1 and PADPA are strong sensitizers in the GPMT. It can be assumed that individuals primarily sensitized to Disperse Orange 1 could react to PADPA, but not to another potential metabolite from azo reduction, that is, 4-nitroaniline, or to Disperse Yellow 3, PPD, 4-aminoacetanilide, or 2-amino-*p*-cresol. Therefore, PPD does not seem to be a suitable marker for the detection of patients who have been primarily sensitized to Disperse Orange 1. Whether Disperse Orange 1 and PADPA cross-react cannot be stated with certainty from the results of this study. It might be that Disperse Orange 1 sensitizes via conversion to PADPA on and/or in the skin, but we cannot, at present, completely rule out the possibility that it sensitizes directly and is cross-reactive with PADPA.

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## References

- Ryberg K, Isaksson M, Gruvberger B, Hindsén M, Zimerson E, Bruze M. Contact allergy to textile dyes in southern Sweden. *Contact Dermatitis* 2006; **54**: 313–321.
- Malinauskiene L, Zimerson E, Bruze M, Ryberg K, Isaksson M. Patch testing with the textile dyes Disperse Orange 1 and Disperse Yellow 3, some of their potential metabolites and simultaneous reactions to para-amino compounds. *Contact Dermatitis* 2012; **67**: 130–140.
- Goon A T, Gilmour N J, Basketter D A, White I R, Rycroft R J, McFadden J P. High frequency of simultaneous sensitivity to Disperse Orange 3 in patients with positive patch tests to para-phenylenediamine. *Contact Dermatitis* 2003; **48**: 248–250.
- Seidenari S, Mantovani L, Manzini B M, Pignatti M. Cross-sensitization between azo dyes and para-amino compound. A study of 236 azo-dye-sensitive subjects. *Contact Dermatitis* 1997; **36**: 91–96.
- Stingley R L, Zou W, Heinze T M, Chen H, Cerniglia C E. Metabolism of azo dyes by human skin microbiota. *J Med Microbiol* 2010; **59**: 108–114.
- Collier S W, Storm J E, Bronaugh R L. Reduction of azo dyes during in vitro percutaneous absorption. *Toxicol Appl Pharmacol* 1993; **118**: 73–79.
- Andersen K E, Bruze M, Karlberg A T, Wahlberg J E, Volund A. How to do sensitization tests in guinea pigs. *Contact Dermatitis* 1994; **31**: 278–279.
- Ryberg K, Gruvberger B, Zimerson E et al. Chemical investigations of disperse dyes in patch test preparations. *Contact Dermatitis* 2008; **58**: 199–209.
- Bruze M. Contact sensitizers in resins based on phenol and formaldehyde. *Acta Derm Venereol Suppl (Stockh)* 1985; **119**: 1–83.
- Magnusson B, Kligman A M. *Allergic Contact Dermatitis in the Guinea Pig. Identifications of Contact Allergens*: Springfield, IL, Charles C. Thomas, 1970.
- Wahlberg J E, Boman A. Guinea pig maximization test. In: *Contact Allergy Predictive Test in Guinea Pigs*, Andersen K E, Maibach H I (eds): Basel, S. Karger AG, 1985: pp. 59–106.
- Pontén A, Zimerson E, Bruze M. Sensitizing capacity and cross-reactivity of phenyl glycidyl ether studied in the guinea-pig maximization test. *Contact Dermatitis* 2009; **60**: 79–84.
- Bruze M, Dahlquist I, Fregert S, Gruvberger B, Persson K. Contact allergy to the active ingredients of Kathon CG. *Contact Dermatitis* 1987; **16**: 183–188.
- Ryberg K, Goossens A, Isaksson M, Gruvberger B, Zimerson E, Persson L, Bruze M. Patch testing of patients allergic to Disperse Blue 106 and Disperse Blue 124 with thin-layer chromatograms and purified dyes. *Contact Dermatitis* 2009; **60**: 270–278.
- Malinauskiene L, Zimerson E, Bruze M, Ryberg K, Isaksson M. Textile dyes Disperse Orange 1 and Yellow 3 contain more than one allergen as shown by patch testing with thin-layer chromatograms. *Dermatitis* 2011; **22**: 335–343.
- Thyssen J P, Gimenez-Arnau E, Lepoittevin J P, Menné T, Boman A, Schnuch A. The critical review of

- methodologies and approaches to assess the inherent skin sensitization potential (skin allergies) of chemicals. Part I. *Contact Dermatitis* 2012; **66**: 11–24.
- 17 Hausen B M, Sawall E M. Sensitization experiments with textile dyes in guinea pigs. *Contact Dermatitis* 1989; **20**: 27–31.
  - 18 Dinardo J, Draelos Z D. An animal model assessment of common dye-induced allergic contact dermatitis. *J Cosmet Sci* 2007; **58**: 209–214.
  - 19 Stahlmann R, Wegner M, Riecke K, Kruse M, Platzek T. Sensitizing potential of four textile dyes and some of their metabolites in a modified local lymph node assay. *Toxicology* 2006; **219**: 113–123.
  - 20 Betts C J, Dearman R J, Kimber I, Maibach H I. Potency and risk assessment of a skin-sensitizing disperse dye using the local lymph node assay. *Contact Dermatitis* 2005; **52**: 268–272.
  - 21 Kimber I, Maibach H I, Msotschi H. Thresholds of contact sensitization from disperse dyes in textiles. *Contact Dermatitis* 2005; **52**: 295.
  - 22 Ahuja V, Platzek T, Fink H, Sonnenburg A, Stahlmann R. Study of the sensitising potential of various textile dyes using a biphasic murine local lymph node assay. *Arch Toxicol* 2010; **84**: 709–718.
  - 23 Hatch K L, Maibach H I. Textile dye allergic contact dermatitis prevalence. *Contact Dermatitis* 2000; **42**: 187–195.
  - 24 Sonnenburg A, Ahuja V, Schreiner M, Platzek T, Stahlmann R. Assessment of the sensitizing potential of textile disperse dyes and some of their metabolites by the loose-fit coculture-based sensitization assay (LCSA). *Arch Toxicol* 2012; **86**: 733–740.
  - 25 Yamano T, Shimizu M. Skin sensitization potency and cross-reactivity of p-phenylenediamine and its derivatives evaluated by non-radioactive murine local lymph node assay and guinea-pig maximization test. *Contact Dermatitis* 2009; **60**: 193–198.
  - 26 Pontén A, Zimerson E, Sörensen O, Bruze M. Sensitizing capacity and cross-reaction pattern of the isomers of diglycidyl ether of bisphenol F in the guinea pig. *Contact Dermatitis* 2002; **47**: 293–298.
  - 27 Zimerson E, Bruze M. Sensitizing capacity of 5,5'-di-tert-butyl-2,2'-dihydroxy-(hydroxymethyl)-dibenzyl ethers in the guinea pig. *Contact Dermatitis* 2000; **43**: 72–78.
  - 28 Kleniewska D, Maibach H. Allergenicity of aminobenzene compounds: structure-function relationships. *Dermatosen* 1980; **28**: 11–13.
  - 29 Benezra C, Maibach H. True cross-sensitization, false cross-sensitization and otherwise. *Contact Dermatitis* 1984; **11**: 65–69.
  - 30 Bruze M, Zimerson E. Cross-reaction patterns in patients with contact allergy to simple methylol phenols. *Contact Dermatitis* 1997; **37**: 82–86.
  - 31 Ryberg K, Goossens A, Isaksson M et al. Is contact allergy to disperse dyes and related substances associated with textile dermatitis? *Br J Dermatol* 2009; **160**: 107–115.
  - 32 Koopmans A K, Bruynzeel D P. Is PPD a useful screening agent? *Contact Dermatitis* 2003; **48**: 89–92.
  - 33 Seidenari S, Giusti F, Massone F, Mantovani L. Sensitization to disperse dyes in a patch test population over a five-year period. *Am J Contact Dermat* 2002; **13**: 101–107.
  - 34 The Hazardous Substances Data Bank (HSDB), a database of the National Library of Medicine's TOXNET system. p-Aminodiphenylamine. Available at: <http://toxnet.nlm.nih.gov> (last accessed 05 September 2012).
  - 35 Foti C, Elia G, Filotico R, Angelini G. Purpuric clothing dermatitis due to Disperse Yellow 27. *Contact Dermatitis* 1998; **39**: 273.
  - 36 Dawes-Higgs E, Freeman S. Allergic contact dermatitis caused by the clothing dye, disperse blue 106, an important contact allergen that may be frequently missed. *Australas J Dermatol* 2004; **45**: 64–66.