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CONTACT DERMATITIS

# Patch test dose–response study of *p*-phenylenediamine: thresholds and anatomical regional differences

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The elicitation response in allergic contact dermatitis for the hair dye substance *p*-phenylenediamine (PPD) is dose dependent, but threshold concentrations have not previously been investigated. 15 PPD-sensitive patients participated in a serial dilution 48-hr patch test with PPD using 8 concentrations of PPD ranging from 1 to 10 000 on the upper back. Petrolatum was applied as control. Three concentrations (50, 100 and 500 p.p.m. PPD) were also applied to the retroauricular area and on the lateral aspects of the upper arms. 14 of the 15 participants reacted to one or more of the test samples. The threshold value for 10% of the tested persons ($ED_{10}$) based on + or stronger reactions for PPD on the back was 38 p.p.m. (CI: 4.3–100). There were no statistically significant differences in the sensitivity of the three anatomical regions. The upper back is a suitable region for patch testing patients with hair dye dermatitis.

*Key words:* allergy; contact dermatitis; cosmetics; dose–response; hair colourant; hair dyes; legislation; para-phenylenediamine; patch test; patients; PPD; *p*-phenylenediamine; regional differences; retroauricular; risk assessment; thresholds; upper arm. © Blackwell Munksgaard, 2006.

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*p*-Phenylenediamine (PPD) has been used as a hair colourant for approximately 100 years and is the second most used dye component after toluene-2,5-diamine in consumer and professional products for hair colouring (1). PPD is one of the most potent contact allergens, based on tests in laboratory animals and human clinical experience (2,3). As a cosmetic ingredient, PPD is permitted in oxidative hair dyes up to a concentration of 6% (60 000 p.p.m.) (4). Youngsters' use of hair dyes is increasing (5), and adverse skin reactions compatible with allergic contact dermatitis have been reported by a significant number of those dying their hair (6). In a recent study, 5.3% of a general population sample reported symptoms: redness, scaling and itching or oedema following hair dying (7). Some of these reactions occur in children; they are severe and necessitate hospitalization and acute treatment (6,8,9). Knowledge of dose–response relationships of hair dye allergens such as PPD is pivotal for risk assessment and re-evaluation of the safety of hair dye ingredients.

This study was undertaken to evaluate the dose-effect elicitation of allergic contact dermatitis in PPD allergic patients with 48-hr patch testing. Threshold levels and anatomical regional differences were recorded.

## Materials and Methods

### *Subjects*

Candidates for testing were eczema patients 18 years or above who had a positive patch test reaction to PPD in the period 1999–2003 at the Department of Dermatology, Gentofte Hospital, Denmark. The exclusion criterion was active eczema. The study included 15 PPD allergic patients: 1 man and 14 women. The local Ethical Committee for Copenhagen approved the protocol with no. KA 04092, and informed consent was obtained from all participants before inclusion in the study. The study ran from January to July 2005.

### *Test substances*

The test material was the standard patch test concentration for PPD, 1% (10.000 p.p.m.) in white petrolatum (pet.) from Hermal Trolab (Reinbek,

Germany). As PPD is unstable in water/ethanol, the samples were diluted in white pet. The serial dilution had concentrations ranging from 1 to 10.000 p.p.m. White pet. was applied as control. To minimize the influence of oxidation, we made the dilutions every week and stored below 5°C.

*Test method and assessment of reactions*

Patch testing was performed with 8-mm Finn Chambers (Epitest Ltd Oy, Tuusula, Finland) on Scanpor® tape, Alpharma AS, Norway, using 20 mg of each patch test material. The dose per unit area was 400 μg/cm$^2$ for 1% PPD. The chambers remained in place for 48 h, and readings were made on D3 and D7 according to the International Contact Dermatitis Group's (ICDRG) scale of reading (10): a + reaction was defined as homogenous redness and infiltration covering the whole test area; +? were responses less than this. To enable statistical calculations, patch test reactions were scored as follows: negative = 0; doubtful (+?) = 1; weakly positive (+) = 2; moderately positive (++) = 3 and strongly positive (+++) = 4. The test preparations on the back were placed with the four highest concentrations on the left and the following four and the control on the right of the back. Samples (500, 100, 50 p.p.m. PPD and pet.) were also placed on the outer aspects of the upper arms and retroauricular area, in a randomized order, with two samples next to each other on each arm and behind each ear. The randomization on the arms was identical to that behind the ears. The test preparations behind the ears were placed just below the hairline (Fig. 1).

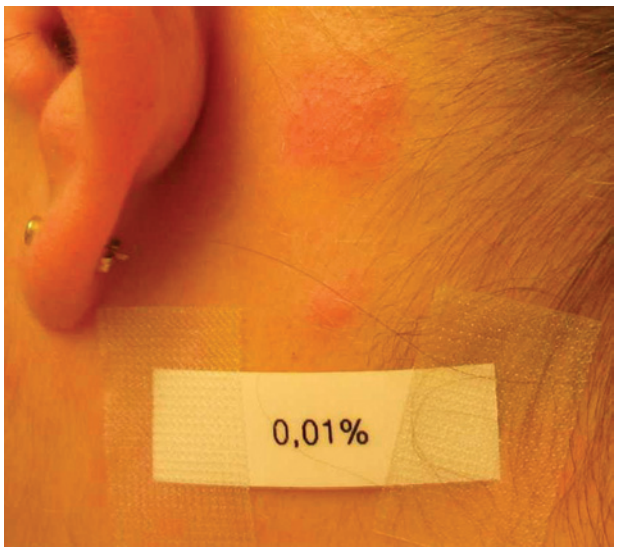


*Fig. 1.* D3 after 48 hours occluded patch test with 0.01% (bottom) 0.05% (top) p-phenylenediamine in petrolatum. The upper patch were read to give a ++ reaction and the lowest patch a + reaction according to the ICDRG scale.

*Statistical methods*

Response data are presented as the percentage of positive patients of a total of 15, and the probability for positive response $P(x)$ is described by the logistic regression model. The probability of positive response by a given dose $x$ is as follows:

$$P(x) = \frac{x^{\lambda}}{ED_{50}^{\lambda} + x^{\lambda}}$$

$ED_{50}$ is the concentration where $P = 0.5$, showing that 50% of the patients will have an allergic reaction; λ is a positive constant showing the steepness of the dose–response curve at $ED_{50}$. As it is the same 15 patients who were tested with all the doses, $P(x)$ is a probability distribution of the threshold dose. One patient had a +? behind the ear on pet. and a negative response to 50 p.p.m. PPD. In the analysis used, the positive reaction to dose = 0 was withdrawn. The hypothesis $\lambda = 0$ corresponds to no statistically significant effect of dose. These hypotheses are rejected in all cases. A $P$ value is given for goodness of fit, meaning if the logistic regression model can describe data except from random deviations. Dose–response curves were drawn for each of the three anatomical regions. These are the dose–response curves that fit best to the observed response frequencies according to the maximum likelihood principle (Fig. 2).

McNemar's test and Wilcoxon's test (11) were used to test for regional differences in responses between ears, arms and back.

## Results

1 man and 14 women aged 24–64 years participated in the study. 1 woman gave no response to PPD despite having had a ++ reaction 6 years previously. All the others gave at least +?

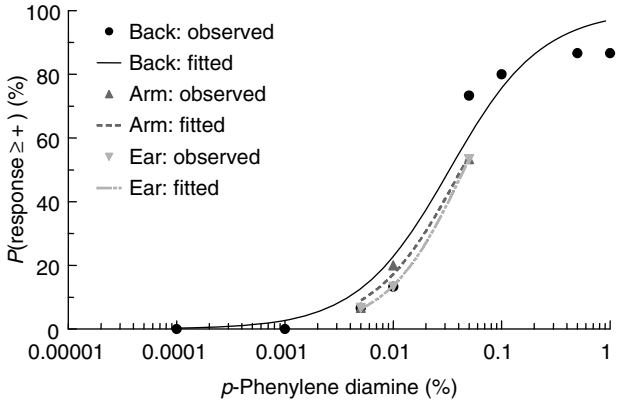


*Fig. 2.* Observed and fitted dose–response curves. Exposure dose and observed response $P$(response) in 15 patients. All patients had a 48-hr patch test read on day 2, 3 and 7. Observed responses were + or stronger.

reaction to PPD 1% in pet.; 7 of the 15 had other contact allergies (fragrance, methyldibromo glutaronitrile, tert-butylphenol formaldehyde resin, disperse orange, thioglycol acid and ammonium persulphate). None of the participants had ever had a semipermanent tattoo.

12 of the participants have previously coloured their hair. The mean number of hair colourings performed in lifetime was 113 (range 8–222), and 10 participants had had a previous allergic reaction to a hair dye product or an allergic reaction to a dye product for eyelashes and brows. Results of 48-hr patch testing with PPD dilution are given in Table 1. There were no differences in the sensitivity (mean SUM score) between the group with a hair dye reaction, $n = 12$, and those with no reaction, $n = 3$ (data not shown).

The threshold value $ED_{10}$ based on + responses or more on the back was 38 p.p.m. (CI: 4.3–100) (Table 2). For each of the three regions (back, ears and arms), dose–response curves were drawn (Fig. 2). The threshold value for 10% of those tested ($ED_{10}$) based on +? responses or more on the back was 4.7 p.p.m. (CI: 0.31–17 p.p.m.), data not shown. McNemar's test and Wilcoxon's test comparing the response at the three concentrations, which were applied on the arms, back and behind the ears showed that there were no regional differences (data not shown). The mean SUM score behind the ears for persons with a previous allergic reaction to a hair dye product (2.0) was not significantly higher than mean SUM score in the same group on the arms (2.2) and on the back (3.1). The participants reported that hair washing had been difficult during the trial, and some had cosmetic disadvantages while the patches were placed behind their ears.

*Table 1*. Results of 48-hr patch testing with *p*-phenylenediamine (PPD)

| PPD concentration (p.p.m.) | Back reactions (%) | Ear reactions (%) | Arm reactions (%) |
|---|---|---|---|
| 1 | 0 | | |
| 10 | 0 | | |
| 50 | 7 | 7 | 7 |
| 100 | 13 | 13 | 20 |
| 500 | 73 | 53 | 53 |
| 1000 | 80 | | |
| 5000 | 87 | | |
| 10 000 | 87 | | |

The table shows the proportion of 15 PPD-allergic individuals who gave positive patch test reactions to treatment with doses of PPD (1–10 000 p.p.m.) applied on different anatomical regions. 1 p.p.m. is 0.0001%. Blank means not tested. No 1+ reactions were recorded to petrolatum.

## Discussion

We investigated the thresholds and anatomical regional differences of PPD in 15 allergic patients.

PPD is permitted in cosmetic products in a concentration of 60 000 p.p.m. (6%), which makes it sensible to compare exposures from different studies in p.p.m. In the 1960s and 1970s, induction and elicitation studies were made with 100–1000 p.p.m. PPD on healthy volunteers (12). At 100 p.p.m., 7.2% of test persons were sensitized and 53% at exposure to 10 000 p.p.m. PPD (13). Today, a typical dark hair dye product may contain 2% PPD (14), a concentration that based on the available knowledge gives a significant risk of sensitization. Elicitation dose-time relationship with PPD has been reported in concentrations ranging from 100 to 10 000 p.p.m. (15). Reactions to 3000 p.p.m. were seen after 5 min (16), while 100 p.p.m. elicited allergic reactions after 120 min (15), but lower concentrations were not investigated. Hair dye products, which have elicited allergic reactions, have contained 17 000 p.p.m. and 2700 p.p.m. PPD (17,18). A use test thresholds study with hair dye products containing precursor, coupler and oxidator has not been carried out yet. In our study, the threshold for + reaction in 10% of the participants was 38 p.p.m. PPD (CI: 4.3–100) assessed as the reactions to a 48-h patch test. Krasteva M et al. (14) investigated permanent hair dye products (without oxidation with hydrogen peroxide) containing PPD that were applied to 34 PPD-positive hair dye allergic individuals in an open test placed to the retroauricular area. 79% reacted to 1000 p.p.m. (compared to 80% in our 48-hr occluded patch test study), 88% reacted to 5000 p.p.m. (compared to 87% in our study) and 97% reacted to 10 000 p.p.m. PPD (compared to 87% in our study). The reactions with a hair dye formulation in an open test are very similar to those of our 48-hr patch test with PPD only. Because Krasteva et al. (14) did not test lower than 1000 p.p.m. PPD, no conclusions can be drawn about reactions to lower concentrations in an open test.

A study with real hair dying would need new product samples for each concentration to ensure that the study described real-life situations. The allergenicity of the coupler, e.g. *p*-aminophenol, could disturb the picture of the sensitivity of PPD. Our study with PPD alone without coupler provides a realistic impression of the threshold where PPD can be applied without inducing symptoms in allergic persons. A study with patients allergic to the couplers is needed.

*Table 2.* Calculated values of $ED_{xx}$ p.p.m. *p*-phenylenediamine with 95% confidence intervals (CI) for reactions + or stronger

| | Back | | Ear | | Arm | |
|---|---|---|---|---|---|---|
| | Value (p.p.m.) | 95% CI (p.p.m.) | Value (p.p.m.) | 95% CI (p.p.m.) | Value | 95% CI (p.p.m.) |
| $ED_{10}$ | 38 | 4.3–100 | 75 | 2.6–180 | 56 | 1.8–150 |
| $ED_{25}$ | 110 | 27–270 | 180 | 30–400 | 150 | 24–380 |
| $ED_{50}$ | 330 | 130–950 | 450 | 190–1700 | 430 | 160–1900 |
| $ED_{75}$ | 970 | 400–5100 | 1100 | 490–17 000 | 1200 | 460–22 000 |
| $ED_{90}$ | 2800 | 980–34 000 | 2700 | 950–220 000 | 3200 | 1000–350 000 |

1 p.p.m. is 0.0001%.

In the 1960s, Magnusson and Hersle (19) showed that allergic skin reactions after 24-hr patch tests were significantly stronger on the back than those on the extremities. Site variations in patch test responses on the upper and lower back have also been shown with laser Doppler flowmetry; however, the differences were insignificant with visual reading (20). In the present study, there are no statistical differences between the three regions: retroauricular area, upper arms and back. Accordingly, PPD applied on the back gives a representative response of the sensitivity of the regions on the arm and behind the ears. 10 of the 15 patients who had had a previous allergic reaction to a hair dye product did not react more strongly behind the ears than on the back and arm. Studying dose–response relationships on the back is technically easier and more cosmetically acceptable to the patient than the retroauricular.

As most of the used hair dye ingredients are predicted to be strong/moderate sensitizers (21), substitution to other dyes is not easy. In prevention of hair dye allergy, it might be necessary to reduce the content of dye allergen to a concentration where it is tolerable for allergic patients. In this study, the elicitation threshold value for 10% of the patch-tested persons ($ED_{10}$) on the back was 38 p.p.m. *p*-phenylenediamine. In studies of permanent hair dye allergic contact dermatitis among children and adults, we found the elicitation and/or induction concentration for PPD and its derivatives in products in a range from 100 p.p.m. to 39 900 p.p.m. A group of control products randomly collected showed a similar concentration range (6,17).

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