



## ORIGINAL ARTICLE

# Twenty-eight-day follow-up of patch test reactions to p-phenylenediamine and p-phenylenediamine dihydrochloride: A multicentre study on behalf of the European Environmental and Contact Dermatitis Research Group

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**Background:** There is an ongoing discussion on whether routinely patch testing with p-phenylenediamine (PPD) 1.0% pet. is safe, owing to the risk of patch test sensitization. Late-appearing patch test reactions may reflect patch test sensitization, but may also be attributable to a low degree of pre-existing sensitization.

**Objectives:** To follow the positive patch test reactions to PPD and its salt PPD dihydrochloride (PPD-DHC) in order to characterize reaction patterns concerning time and dose in PPD-sensitized individuals.

**Methods:** Volunteers with previous reactions to PPD 1.0% were included and patch tested with PPD and PPD-DHC in equimolar dilution series. There were then seven follow-up visits over a period of 28 days.

**Results:** Twenty-six volunteers completed the study, of whom 23 of 26 (88%) reacted to PPD 1.0%, and 69% reacted to PPD 0.32%. Altogether, 42% and 27% reacted to the corresponding equimolar concentrations of PPD-DHC. After day 7, no new reactions were observed to any concentration tested, either of PPD or of PPD-DHC.

**Conclusion:** No late-appearing reactions to PPD or PPD-DHC were observed at any dose. There is a risk of missing contact allergy when the dose is decreased.

**KEYWORDS**

active sensitization, allergic contact dermatitis, contact allergy, delayed, dilution series, false-negative reactions, hypersensitivity, late-appearing patch test reactions, PPD

This work corresponds to parts of the PhD thesis "Oxidized p-Phenylenediamine in Contact Allergy—Clinical and Experimental Studies", Ewa Young, Lund University, 2018. The thesis is available at [http://portal.research.lu.se/ws/files/49558204/Thesis\\_Oxidized\\_PPD\\_Young\\_2018.pdf](http://portal.research.lu.se/ws/files/49558204/Thesis_Oxidized_PPD_Young_2018.pdf)

Abbreviations: D, day; DPCP, diphenylcyclopropenone; PPD, p-phenylenediamine; PPD-DHC, p-phenylenediamine dihydrochloride.

## 1 | INTRODUCTION

p-Phenylenediamine (PPD) is a potent contact sensitizer and a common constituent of permanent hair dyes. The sensitizing capacity of PPD has been known for >100 years. As early as 1939, PPD was suggested for screening for hair dye allergy, and was therefore included in one of the early baseline series for patch testing at 2% (pet.).<sup>1</sup> In the ICDRG baseline series dated 1974, PPD was included at 1% pet.<sup>2</sup> The

recommended routine patch test concentration of PPD for diagnosing contact allergy in sensitized patients has been 1.0% pet., and PPD 1.0% pet. is currently still included in the European baseline series.<sup>3</sup>

For over a decade, it has been discussed whether PPD 1% pet. is safe for routine patch testing, owing to the risk of active sensitization, that is, sensitization of hitherto non-sensitized patients by the patch test itself, and whether or not the dose used in the patch test should be reduced for this reason.<sup>4–17</sup> In this study, the aim was to investigate the occurrence of late-appearing reactions in already sensitized individuals when these cannot be explained by active sensitization.

When the patch test statistics of the German Information Network of Departments of Dermatology in the years 1995 to 2004 were analysed, 4% of 83 030 individuals tested were PPD-positive.<sup>8</sup> In this patient population, a high proportion (44%) of PPD reactions without any known current relevance was found.<sup>8</sup> Hillen et al<sup>14</sup> performed prospective investigations in order to study late-occurring reactions in routine patch testing with PPD 1% pet. Readings were performed on day (D) 3, D7, D14, and D21. The patch tests were placed on the arm, and the patients were told to make daily evaluations and call if they suspected a positive reaction. Among 1428 individuals, 3.2% (n = 46) had positive reactions on D3 and 1.5% (n = 21) had positive reactions on D7 and beyond. Among the 21 patients with late reactions, 3 had de novo reactions on D7 and the remaining 18 had positive reactions appearing at readings on D11 to D37. In total, 7 of the 21 patients were retested with PPD 1%, and in 5 of 7 there was a positive reaction within 3 days on retesting.<sup>14</sup> On the basis of these results, Hillen et al<sup>14</sup> estimated an active sensitization rate of 1% in patients routinely patch tested with PPD 1.0% pet. applied for 48 hours.

On the basis of these data, removal of PPD 1.0% from the baseline patch test series was recommended in Germany by the German Contact Dermatitis Research Group, with the suggestion of performing only aimed testing.<sup>14</sup> To avoid active sensitization, patch testing was recommended only if there was a clear suspicion of possible allergic contact dermatitis caused by PPD from the patient's medical history and distribution of eczema. Lower patch test concentrations of 0.30% to 0.35% PPD have been proposed.<sup>4,14</sup>

However, delayed patch test reactions may also occur for reasons other than active sensitization, for example, chemical properties and

skin permeation. The drawback of performing only aimed testing is that the patient's history may not show exposure to PPD. Apart from PPD being a substance used for diagnosing contact allergy to hair dyes in general, it is known that several other allergens can cross-react with PPD. Thus, excluding it from the baseline series might lead to missed contact allergy diagnoses in patients who have been sensitized to, for example, hair dyes, black rubber, and textile dyes.

PPD is commonly used in patch testing in its pure form, but, in the period 1984 to 1988 it was included in the baseline series as a salt, PPD dihydrochloride (PPD-DHC).<sup>2</sup> Following a recommendation of the European Environmental and Contact Dermatitis Research Group (EECDRG) and the ICDRG, this was abandoned in favour of going back to testing with PPD, as too many allergies had been missed.<sup>2</sup>

The aim of this study was to follow the morphology of patch test reactions of dilution series of PPD and PPD-DHC in a cohort of individuals who were already sensitized to PPD. The volunteers were seen on seven occasions over a period of 28 days to detect any late-appearing reactions that might be misinterpreted as signs of active sensitization.

## 2 | MATERIALS AND METHODS

The following chemicals were used: PPD (>99%; Sigma-Aldrich, St. Louis, Missouri), PPD-DHC (>99%; Fisher Scientific, Bridgewater, New Jersey), ethanol (>99.5%; CCS Healthcare, Borlänge, Sweden), and distilled water (Q-guard 1; Millipore, Molsheim, France).

PPD and PPD-DHC were dissolved in ethanol/water 70:30 vol/-vol at equimolar concentrations of 1.0% and 1.7% wt/vol, respectively. The pH values of the PPD and PPD-DHC solutions were 8 and 3, respectively. These stock solutions were further diluted in ethanol/water with a dilution factor of  $\sqrt{10}$ , yielding 11 concentrations per chemical (Table 1). Ethanol/water (70:30 vol/vol) was chosen as the vehicle because of good solubility for both PPD and PPD-DHC. All solutions were prepared at the Department of Occupational and Environmental Dermatology in Malmö. The solutions were transported cooled, either by air or by road, to the three participating test centres outside of Sweden. The staff at the participating clinics were instructed to store the solutions in a refrigerator, and to keep track of the preparation date. The test preparation was supposed to be tested within 1 week.

For patch testing, the dilution series of PPD and PPD-DHC were applied on the backs of the volunteers in Finn Chambers (diameter of 8 mm; Epitest, Tuusula, Finland, now SmartPractice, Phoenix, Arizona). A micropipette was used to apply 15  $\mu$ L of each test solution to the test chambers.<sup>18</sup> Each person was patch tested with 11 doses of PPD and of PPD-DHC (Table 1). Patch tests were removed after 2 days, that is, on D2.

Patch tests were evaluated and scored after 2 days (minimum of 30 minutes after patch test removal), and this was followed by readings on D3 or D4, D7, D10, D14, D21, and D28. Patch test reading was performed according to the criteria of the ICDRG.<sup>19</sup> For each volunteer, seven protocols were used, one for each patch test reading. The scoring protocol for PPD and PPD-DHC included a list for scoring

**TABLE 1** The 11 equimolar concentrations of *p*-phenylenediamine (PPD) and *p*-phenylenediamine dihydrochloride (PPD-DHC) that were patch tested

| PPD (% wt/vol) | PPD-DHC (% wt/vol) |
|----------------|--------------------|
| 1.0            | 1.7                |
| 0.32           | 0.53               |
| 0.10           | 0.17               |
| 0.032          | 0.053              |
| 0.010          | 0.017              |
| 0.0032         | 0.0053             |
| 0.0010         | 0.0017             |
| 0.00032        | 0.00053            |
| 0.00010        | 0.00017            |
| 0.000032       | 0.000053           |
| 0.000010       | 0.000017           |

and a list of specified morphological evaluation features such as erythema, infiltration, papules, squamation, and pustules. Each of the 22 test spots was evaluated according to the morphological features, which were marked if present. This morphological evaluation served as an aid for the patch test readers to perform the overall scoring. On the basis of the morphological scoring, each reader of a test made an overall evaluation, with scores of +, ++, +++, doubtful reaction, irritant reaction, and no reaction. This scoring by the reader was then used in further analysis. Treatment with topical corticosteroids was performed if necessary. If treated, the area was not read further.

The inclusion criteria for study participation were an age of at least 18 years and a previous positive test reaction to PPD. In four study centres, dermatitis patients with previous positive reactions to PPD were identified from patch test data records, contacted, and invited to participate either by telephone or by letter. Patch tests were performed in 2011 and 2016 in Malmö, in 2014 in Gentofte and Odense, and in 2015 in Barcelona. Altogether, 34 study subjects were included (6 in Barcelona, 10 in Gentofte, 6 in Odense, and 12 in Malmö). The study was approved by the regional ethics boards in Sweden, Denmark, and Spain. The study was conducted in accordance with the ethical standards specified in the Declaration of Helsinki.

### 3 | RESULTS

Of the 34 study subjects 26 completed the study, that is, they were patch tested with dilution series of PPD and PPD-DHC, and the test area was read seven times according to the protocol as shown in Table 2. Twenty-three of 26 (88%) reacted to PPD 1.0%, whereas 69% reacted to PPD 0.32%. In total, 42% and 27%, respectively, reacted to the corresponding equimolar concentrations of PPD-DHC. No new reactions after day 7 were observed at any concentration tested, either with PPD or with its salt (PPD-DHC). Eight (31%) of the previously PPD-positive test subjects and five (22%) of those who were currently positive for PPD 1.0% were negative on testing with 0.32% PPD on days 2 to 7, whereas the same number ( $n = 17$ ) reacted positively to PPD 1.0% and 0.32% on D2. Reactions that did not appear until D7 occurred in 4 of 26 study subjects (15%; volunteers 17, 19, 22, and 23). This pattern of reactions not appearing until D7 was also seen for 0.32% PPD in study subjects 20 and 23 (2/26) and for 0.1% PPD in study subject 13. In no other volunteer did any new reactions to PPD occur that had not appeared until D7. After D7, there were no new positive patch test reactions to any of the PPD concentrations tested. Figure 1 shows the total number of positive reactions over time for the three highest concentrations of PPD tested. Generally, positive reactions appeared between D2 and D7, and then decreased in intensity. All of these had disappeared by D28.

Fifteen study subjects had positive reactions to PPD-DHC 1.7%. Reactions that did not appear until D7 occurred in 2 of 26 study subjects (8%; volunteers 9 and 11). In no other volunteers did reactions occur to PPD-DHC that did not appear until D7. There were no new positive patch test reactions after D7 to any of the PPD-DHC concentrations tested. In Figure 2, the total number of positive reactions over time is shown for the three highest concentrations of PPD-DHC

tested. Generally, positive reactions appeared on D2 to D7, and then decreased in intensity. All of these had disappeared by D28.

The test areas that were treated with corticosteroid cream, because of extreme reactions, were not evaluated in further readings due to the treatment. This occurred in six study subjects on D2 or D4. In order to include these reactions in our overall analysis of the number of reactions over time, these treated areas were counted as positive up to D14 and negative from D21. This estimation was made in accordance with the trend observed for untreated +++ reactions.

### 4 | DISCUSSION

Active sensitization, which is synonymous with patch test sensitization, is a possible adverse effect of patch testing and represents sensitization caused by the patch test procedure. Patch test sensitization is usually suspected if a positive patch test reaction develops after D7, or particularly after D10 to D14 and beyond. If a subsequent retest with the same preparation gives a positive reaction within 7 days, the initial late-appearing positive reaction is suspected to represent active sensitization.<sup>20,21</sup> In Malmö, retesting is also performed with dilution series of the allergen, on the basis of an article published by Bruze in 1984,<sup>22</sup> although a retest with a dilution series will not always discriminate between patch test sensitization and a late-appearing positive patch test. A late patch test reaction is, however, not always attributable to patch test sensitization.<sup>23–25</sup>

Causes of variations in reaction time and sensitivity to an allergen may be chemical properties, skin permeation, interindividual variation in the expression of involved metabolic enzymes, and an anti-inflammatory influence from the allergen. Repeated topical exposure may affect the level of sensitivity. In an experimental study performed by Mose et al, increased sensitivity was reported between first and second challenges with diphenylcyclopropanone (DPCP) in human volunteers sensitized with DPCP. This is suggestive of a booster effect between the first and second patch tests, and thereafter a plateau level of reactivity following the next four to five challenges with 1-month intervals.<sup>26</sup> Late patch test reactions have been observed for some contact allergens, such as corticosteroids, gold, acrylates, isocyanates, and Compositae allergens.<sup>23,25,27–29</sup>

It has been shown that lower test doses of a contact allergen may elicit late positive reactions. Gold-allergic patients have been patch tested with dilution series of gold sodium thiosulfate, and in 1 of 10 patients the lower test doses elicited positive reactions on D10 and D21, whereas reactions to the highest concentrations developed within 7 days.<sup>23</sup> Thus, there is a possibility, when patch testing is performed with serial dilutions of PPD, that reactions to the lower concentrations might appear later than reactions to the highest concentrations—and that a higher concentration could also yield a later reaction, especially in a patient with lower reactivity who would therefore react only to the highest concentration in a dilution series.

We designed this study to investigate reactions to PPD dilution series over time, and to determine whether we could detect any late reaction first appearing on D10 or later. Equimolar dilution series of PPD and its salt PPD-DHC were tested, and the patch test reactions were observed over time for 4 weeks in 26 already PPD-sensitized

**TABLE 2** Part 1/3. Test results for 26 volunteers tested with *p*-phenylenediamine (PPD) and PPD dihydrochloride (PPD-DHC): PPD 1.0% and 0.32%; PPD-DHC 1.7% and 0.53%<sup>a</sup>. Part 2/3. Test results for 26 volunteers tested with PPD and PPD-DHC: PPD 0.1% and 0.032%; PPD-DHC 0.17% and 0.053%<sup>a</sup>. Part 3/3. Test results for 26 volunteers tested with PPD and PPD-DHC: PPD 0.001% and 0.00032%; PPD-DHC 0.0017% and 0.00053%<sup>a</sup>

| Part 1/3 |     |     |     |     |     |     |     |     |     |     |     |     |     |               |     |     |     |     |     |     |            |     |     |     |     |               |                |     |  |  |  |  |  |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|---------------|-----|-----|-----|-----|-----|-----|------------|-----|-----|-----|-----|---------------|----------------|-----|--|--|--|--|--|
| PPD 1.0% |     |     |     |     |     |     |     |     |     |     |     |     |     | PPD-DHC 1.7%  |     |     |     |     |     |     | PPD 0.32%  |     |     |     |     | PPD-DHC 0.53% |                |     |  |  |  |  |  |
| Patient  | D2  | D4  | D7  | D10 | D14 | D21 | D28 | D2  | D4  | D7  | D10 | D14 | D21 | D28           | D2  | D4  | D7  | D10 | D14 | D21 | D28        | D2  | D4  | D7  | D10 | D14           | D21            | D28 |  |  |  |  |  |
| 1        | +++ | +++ | +   | +   | -   | -   | -   | +++ | +++ | +   | +   | -   | -   | -             | +++ | +++ | +   | +   | -   | -   | -          | +++ | +++ | +   | +   | -             | -              | -   |  |  |  |  |  |
| 2        | ++  | ++  | +++ | +   | +   | -   | -   | ++  | ++  | ++  | +   | -   | -   | -             | ++  | ++  | +++ | +   | +   | +   | -          | +   | +   | ++  | +   | -             | -              | -   |  |  |  |  |  |
| 3        | +++ | C   | C   | C   | C   | C   | C   | +++ | +++ | +++ | -   | -   | -   | -             | +++ | C   | C   | C   | C   | C   | C          | ++  | ++  | -   | -   | -             | -              |     |  |  |  |  |  |
| 4        | +++ | +++ | +   | -   | +   | +   | -   | +   | ++  | -   | -   | -   | -   | -             | +++ | +++ | +   | -   | +   | -   | -          | +   | +   | -   | -   | -             | -              |     |  |  |  |  |  |
| 5        | +++ | C   | C   | C   | C   | C   | C   | +   | ++  | ++  | +   | -   | -   | -             | +++ | C   | C   | C   | C   | C   | C          | -   | +   | -   | -   | -             | -              |     |  |  |  |  |  |
| 6        | ++  | +++ | C   | C   | C   | C   | C   | -   | ++  | +++ | -   | -   | -   | -             | +   | +++ | C   | C   | C   | C   | C          | -   | ++  | +++ | -   | -             | -              |     |  |  |  |  |  |
| 7        | +   | +++ | +++ | -   | -   | -   | -   | ++  | +++ | +++ | +   | -   | -   | -             | +   | +++ | +++ | -   | -   | -   | -          | +   | +++ | +++ | +   | -             | -              |     |  |  |  |  |  |
| 8        | +++ | +++ | C   | C   | C   | C   | C   | +++ | +++ | C   | C   | C   | C   | C             | +++ | +++ | C   | C   | C   | C   | C          | +++ | +++ | C   | C   | C             | C              |     |  |  |  |  |  |
| 9        | ++  | +++ | ++  | +   | -   | -   | -   | +   | ++  | ++  | -   | -   | -   | -             | ++  | +++ | ++  | +   | +   | -   | -          | -   | -   | ++  | -   | -             | -              |     |  |  |  |  |  |
| 10       | +++ | C   | C   | C   | C   | C   | C   | ++  | C   | C   | C   | C   | C   | C             | +++ | C   | C   | C   | C   | C   | C          | ++  | +++ | C   | C   | C             | C              |     |  |  |  |  |  |
| 11       | ++  | ++  | ++  | +   | +   | -   | -   | ++  | ++  | ++  | +   | -   | +   | -             | ++  | ++  | ++  | +   | +   | +   | -          | -   | -   | +   | -   | -             | -              |     |  |  |  |  |  |
| 12       | +++ | C   | C   | C   | C   | C   | C   | +++ | +++ | +   | -   | -   | -   | -             | +++ | C   | C   | C   | C   | C   | C          | ++  | +++ | +   | -   | -             | -              |     |  |  |  |  |  |
| 13       | +   | +++ | ++  | +   | +   | -   | -   | -   | ++  | +   | -   | -   | -   | -             | +   | ++  | +   | -   | -   | -   | -          | -   | -   | -   | -   | -             | -              |     |  |  |  |  |  |
| 14       | ++  | +++ | +++ | +   | -   | -   | -   | -   | ++  | +   | -   | -   | -   | -             | ++  | ++  | ++  | -   | -   | -   | -          | -   | -   | -   | -   | -             | -              |     |  |  |  |  |  |
| 15       | +   | ++  | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -             | +   | ++  | -   | -   | -   | -   | -          | -   | -   | -   | -   | -             | -              |     |  |  |  |  |  |
| 16       | -   | +   | +   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -             | -   | -   | -   | -   | -   | -   | -          | -   | -   | -   | -   | -             | -              |     |  |  |  |  |  |
| 17       | -   | -   | +   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -             | -   | -   | -   | -   | -   | -   | -          | -   | -   | -   | -   | -             | -              |     |  |  |  |  |  |
| 18       | -   | ++  | +   | +   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -             | -   | -   | -   | -   | -   | -   | -          | -   | -   | -   | -   | -             | -              |     |  |  |  |  |  |
| 19       | -   | -   | +   | +   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -             | -   | -   | -   | -   | -   | -   | -          | -   | -   | -   | -   | -             | -              |     |  |  |  |  |  |
| 20       | +   | ++  | ++  | +   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -             | -   | -   | +   | -   | -   | -   | -          | -   | -   | -   | -   | -             | -              |     |  |  |  |  |  |
| 21       | -   | +   | -   | -   | -   | -   | -   | -   | +   | +   | -   | -   | -   | -             | -   | -   | -   | -   | -   | -   | -          | -   | -   | -   | -   | -             | -              |     |  |  |  |  |  |
| 22       | -   | -   | ++  | ++  | -   | -   | -   | -   | -   | -   | -   | -   | -   | -             | +   | ++  | ++  | ++  | +   | +   | +          | -   | -   | -   | -   | -             | -              |     |  |  |  |  |  |
| 23       | -   | -   | ++  | +   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -             | -   | -   | ++  | +   | +   | -   | -          | -   | -   | -   | -   | -             | -              |     |  |  |  |  |  |
| 24-26    | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -             | -   | -   | -   | -   | -   | -   | -          | -   | -   | -   | -   | -             | -              |     |  |  |  |  |  |
| Part 2/3 |     |     |     |     |     |     |     |     |     |     |     |     |     |               |     |     |     |     |     |     |            |     |     |     |     |               |                |     |  |  |  |  |  |
| PPD 0.1% |     |     |     |     |     |     |     |     |     |     |     |     |     | PPD-DHC 0.17% |     |     |     |     |     |     | PPD 0.032% |     |     |     |     |               | PPD-DHC 0.053% |     |  |  |  |  |  |
| Patient  | D2  | D4  | D7  | D10 | D14 | D21 | D28 | D2  | D4  | D7  | D10 | D14 | D21 | D28           | D2  | D4  | D7  | D10 | D14 | D21 | D28        | D2  | D4  | D7  | D10 | D14           | D21            | D28 |  |  |  |  |  |
| 1        | +++ | +++ | +   | +   | -   | -   | -   | +++ | +++ | +   | +   | -   | -   | -             | +++ | ++  | ++  | -   | -   | -   | -          | ++  | ++  | -   | -   | -             | -              | -   |  |  |  |  |  |
| 2        | ++  | ++  | ++  | +   | +   | -   | -   | +   | +   | ++  | +   | -   | -   | -             | ++  | ++  | ++  | +   | -   | -   | -          | +   | +   | ++  | +   | -             | -              | -   |  |  |  |  |  |
| 3        | +++ | +++ | C   | C   | C   | C   | C   | -   | ++  | -   | -   | -   | -   | -             | +++ | +++ | C   | C   | C   | C   | C          | +   | ++  | +   | -   | -             | -              |     |  |  |  |  |  |
| 4        | +   | ++  | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -   | -             | +   | ++  | -   | -   | -   | -   | -          | +   | ++  | -   | -   | -             | -              |     |  |  |  |  |  |
| 5        | ++  | C   | C   | C   | C   | C   | C   | -   | +   | -   | -   | -   | -   | -             | -   | -   | -   | -   | -   | -   | -          | -   | -   | -   | -   | -             | -              |     |  |  |  |  |  |

**TABLE 2** (Continued)

| PPD 0.1%   |    |     |    |     |     |     |     |     |     | PPD-DHC 0.17%   |     |     |     |     |    |    |    |     |     | PPD 0.032%   |     |    |     |    |     |     |     |     |  | PPD-DHC 0.053%   |  |  |  |  |  |  |  |  |  |
|------------|----|-----|----|-----|-----|-----|-----|-----|-----|-----------------|-----|-----|-----|-----|----|----|----|-----|-----|--------------|-----|----|-----|----|-----|-----|-----|-----|--|------------------|--|--|--|--|--|--|--|--|--|
| Patient    | D2 | D4  | D7 | D10 | D14 | D21 | D28 | D2  | D4  | D7              | D10 | D14 | D21 | D28 | D2 | D4 | D7 | D10 | D14 | D21          | D28 | D2 | D4  | D7 | D10 | D14 | D21 | D28 |  |                  |  |  |  |  |  |  |  |  |  |
| 6          | -  | +++ | C  | C   | C   | C   | C   | -   | ++  | ++              | -   | -   | -   | -   | -  | ++ | ++ | +   | -   | -            | -   | -  | +   | ++ | -   | -   | -   | -   |  |                  |  |  |  |  |  |  |  |  |  |
| 7          | +  | +++ | +  | -   | -   | -   | -   | -   | ++  | -               | -   | -   | -   | -   | -  | ++ | -  | -   | -   | -            | -   | -  | -   | -  | -   | -   | -   | -   |  |                  |  |  |  |  |  |  |  |  |  |
| 8          | ++ | ++  | C  | C   | C   | C   | C   | ++  | +++ | C               | C   | C   | C   | C   | +  | ++ | C  | C   | C   | C            | C   | ++ | ++  | C  | C   | C   | C   | C   |  |                  |  |  |  |  |  |  |  |  |  |
| 9          | +  | +++ | +  | -   | -   | -   | -   | -   | -   | -               | -   | -   | -   | -   | -  | -  | -  | -   | -   | -            | -   | -  | -   | -  | -   | -   | -   | -   |  |                  |  |  |  |  |  |  |  |  |  |
| 10         | ++ | ++  | +  | -   | -   | -   | -   | ++  | +++ | C               | C   | C   | C   | C   | +  | ++ | -  | -   | -   | -            | -   | +  | ++  | +  | -   | -   | -   | -   |  |                  |  |  |  |  |  |  |  |  |  |
| 11         | +  | +   | +  | -   | -   | -   | -   | -   | -   | -               | -   | -   | -   | -   | +  | +  | +  | -   | -   | -            | -   | -  | -   | -  | -   | -   | -   | -   |  |                  |  |  |  |  |  |  |  |  |  |
| 12         | ++ | +++ | C  | C   | C   | C   | C   | +++ | +++ | -               | -   | -   | -   | -   | ++ | ++ | -  | -   | -   | -            | -   | +  | +++ | -  | -   | -   | -   | -   |  |                  |  |  |  |  |  |  |  |  |  |
| 13         | -  | -   | +  | -   | -   | -   | -   | -   | -   | -               | -   | -   | -   | -   | -  | -  | -  | -   | -   | -            | -   | -  | -   | -  | -   | -   | -   | -   |  |                  |  |  |  |  |  |  |  |  |  |
| 14         | -  | -   | -  | -   | -   | -   | -   | -   | -   | -               | -   | -   | -   | -   | -  | -  | -  | -   | -   | -            | -   | -  | -   | -  | -   | -   | -   | -   |  |                  |  |  |  |  |  |  |  |  |  |
| 15         | -  | ++  | -  | -   | -   | -   | -   | -   | ++  | -               | -   | -   | -   | -   | -  | -  | -  | -   | -   | -            | -   | -  | -   | -  | -   | -   | -   | -   |  |                  |  |  |  |  |  |  |  |  |  |
| 16-26      | -  | -   | -  | -   | -   | -   | -   | -   | -   | -               | -   | -   | -   | -   | -  | -  | -  | -   | -   | -            | -   | -  | -   | -  | -   | -   | -   | -   |  |                  |  |  |  |  |  |  |  |  |  |
| Part 3/3   |    |     |    |     |     |     |     |     |     |                 |     |     |     |     |    |    |    |     |     |              |     |    |     |    |     |     |     |     |  |                  |  |  |  |  |  |  |  |  |  |
| PPD 0.001% |    |     |    |     |     |     |     |     |     | PPD-DHC 0.0017% |     |     |     |     |    |    |    |     |     | PPD 0.00032% |     |    |     |    |     |     |     |     |  | PPD-DHC 0.00053% |  |  |  |  |  |  |  |  |  |
| Patient    | D2 | D4  | D7 | D10 | D14 | D21 | D28 | D2  | D4  | D7              | D10 | D14 | D21 | D28 | D2 | D4 | D7 | D10 | D14 | D21          | D28 | D2 | D4  | D7 | D10 | D14 | D21 | D28 |  |                  |  |  |  |  |  |  |  |  |  |
| 1          | ++ | -   | -  | -   | -   | -   | -   | +   | -   | -               | -   | -   | -   | -   | ++ | -  | -  | -   | -   | -            | -   | -  | -   | -  | -   | -   | -   | -   |  |                  |  |  |  |  |  |  |  |  |  |
| 2          | -  | +   | +  | -   | -   | -   | -   | -   | -   | -               | -   | -   | -   | -   | -  | -  | -  | -   | -   | -            | -   | -  | -   | -  | -   | -   | -   | -   |  |                  |  |  |  |  |  |  |  |  |  |
| 3-26       | -  | -   | -  | -   | -   | -   | -   | -   | -   | -               | -   | -   | -   | -   | -  | -  | -  | -   | -   | -            | -   | -  | -   | -  | -   | -   | -   | -   |  |                  |  |  |  |  |  |  |  |  |  |

Abbreviations: C, cortisone cream treatment; +, weak positive reaction; ++, strong positive reaction; +++, extreme reaction.

D4 readings were performed on either D3 or D4. The patch test on volunteer 19 was read on D9 instead of on D10, and on D29 instead of on D28. Among the three lowest concentrations tested for PPD and PPD-DHC not presented in this table, volunteer 1 had + reactions to 0.0001% and 0.000032% PPD.

<sup>a</sup>Doubtful reactions are replaced with a negative test result.

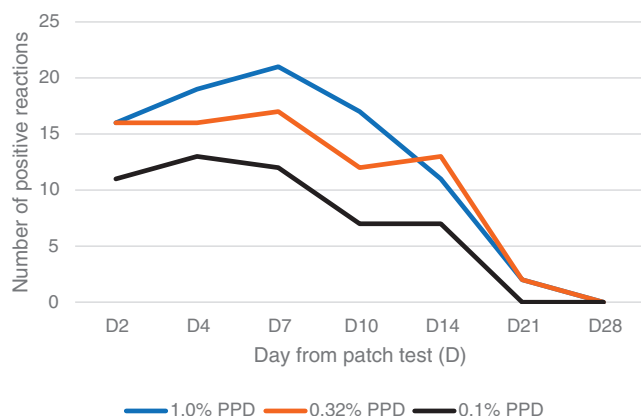


**FIGURE 1** Number of positive reactions to *p*-phenylenediamine (PPD) over time

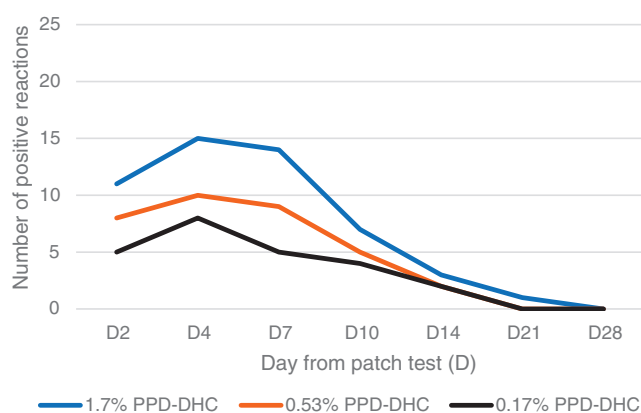


**FIGURE 2** Number of positive reactions to *p*-phenylenediamine dihydrochloride (PPD-DHC) over time

study subjects. None of the 26 study subjects had late-appearing reactions to PPD after D7 (Table 2). We did not see any late-appearing reactions to PPD-DHC either, although, owing to its high polarity, this substance can be expected to have slower skin penetration and thus later elicitation. In the present study, we were unable to show that slower penetration or lower concentrations would lead to new reactions after D7. However, lower concentrations produced later reactions in some cases. This concerned three volunteers with new reactions at reading D7 (for PPD in subject 20 and for PPD-DHC in subjects 9 and 11). When we compared D2 with D4, we saw new reactions developing at D4, to lower concentrations in eight cases (for PPD in subjects 2, 5, 6, 7, and 15, and for PPD-DHC in subjects 3, 5, and 7).

According to Wilkinson et al, the definition of a late reaction is "the reaction occurring at the site of a patch test on day 7 (D7) or later, with no preceding reaction on days 1–6 (D1–D6)".<sup>30</sup> If reactions appearing on D7 were considered to be late reactions, 12% (3/26) of the volunteers in the present study would have late reactions. However, there are different opinions on the definition of a late reaction. At the Department of Occupational and Environmental Dermatology in Malmö, Sweden, D3 and D7 readings are always performed to evaluate clinical patch tests, and a late reaction is defined as a positive reaction occurring after D7.

Hillen et al<sup>14</sup> reported a rate of 1.5% (21/1428) delayed PPD reactions, but patients who were positive on D7 were included. If a late patch test reaction is defined as a positive reaction after D7, the rate of late reactions becomes 1.3% in the Hillen study population, which is still a high figure concerning possible active sensitization. The data presented by Hillen et al together with other PPD patch test data constituted the basis for the recommendation in Germany in 2006 to stop routine patch testing with PPD 1.0% pet., with a subsequent recommendation to lower the patch test concentration to 0.3% pet., to reduce the risk of active sensitization. Others have questioned these findings, stipulating that the risk of active sensitization has been overestimated, and have advocated continuing to use PPD 1% pet. for routine patch testing to avoid missing contact allergy to PPD.<sup>5,13,16,31</sup>

Our results neither support nor contradict the removal of PPD 1% from the routine patch test setup. It is possible that delayed patch test reactions to PPD would occur at a low frequency in a larger cohort of PPD-positive individuals. This could not be confirmed in the present study.

The time for patch test reading varies between different patch test centres. Some late reactions, regardless of the cause, will be missed if patch test reading is performed before the positive patch test reaction has appeared. Reading only on D2 in this study would have missed 10 of 26 (38%) of the previously PPD-positive individuals, or 7 of 23 (30%) of the currently positive test subjects. In this study, 4 of 23 study subjects did not show positive reactions to 1.0% PPD until D7. This means that if the last reading had been on D4, we would have missed 17% of the sensitized volunteers.

Geier et al recommend replacing PPD 1.0% pet. in the baseline patch test series with PPD 0.3% pet.<sup>4</sup> Also, removal of PPD from the baseline series has been suggested, with the recommendation to test with it only when there is a direct suspicion of contact allergy to PPD, in order to avoid patch test sensitization.<sup>17</sup> In this study, 23 of 26 study subjects reacted to PPD 1.0%, whereas 18 of 26 reacted to 0.32% PPD. Lowering the routine patch test concentration to 0.3% might have resulted in the diagnosis of contact allergy to PPD being missed in five study subjects (22%) among those who showed reactions, and in eight study subjects (31%) among those who were previously found to be PPD-positive. Geier et al<sup>4</sup> reported that only 74% of the patients (91/123) who reacted positively to PPD 1% pet. also reacted to PPD 0.3%. The results from testing PPD in pet. presented by Geier are very similar to the results in the present study based on alcoholic solutions of almost the same PPD concentrations 0.32% and 1.0% yielding 78% (18/23) positive reactions. A defined dose per unit area rather than concentration is crucial when the risk of patch test sensitization is evaluated. The elicitation and sensitization capacity depend not only on the concentration, but predominantly on the dose per unit area of allergen delivered to the skin and exposure time.<sup>3,32</sup> In a recent study, good agreement was shown for PPD patch test preparations with equivalent doses of 1.0% and 0.3% pet.<sup>33</sup> If dosed correctly, PPD 1% pet. may be safe to patch test with.

The scoring criteria are adapted to patch test reactions that become positive within 7 days. Here, we followed the reactions until D28. During the healing process, the appearance of the positive reaction site could change to show a morphology that looked more like an

irritant reaction. In the summing-up of the test results, the overall scoring of the test readers was used.

In conclusion, none of the 26 test subjects in the present study showed late-appearing reactions to any concentration tested, either with the base or with the salt. Even though late reactions to PPD that are attributable to causes other than patch test sensitization might occur, they appear to be infrequent. Chaudhry et al<sup>34</sup> conducted a test reading once some time between day 7 and day 14, and found no new positive reactions to PPD. Aalto-Korte et al reported late reactions in a sample of 826 patients tested with PPD, on day 10 to day 14, in 6 patients,<sup>35</sup> who then reacted early on retesting. The risk of patch test sensitization must be considered when routine patch testing with 1.0% PPD is performed. On the other hand, the risk of missed contact allergy must also be considered if the test concentration of PPD is reduced, or if PPD is excluded altogether from routine patch testing. In this study, testing with 0.32% PPD at the most would have resulted in false-negative reactions in eight (31%) of the test subjects who previously reacted positively to PPD.

In this study, late-appearing reactions as a part of sensitization existing before the patch testing could not be shown. However, the number of tested individuals was low. Lowering the patch test concentration will substantially increase the number of false-negative reactions.

## CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

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