28 days follow up of patch-test reactions to p-phenylenediamine and p-phenylenediamine dihydrochloride: a multicenter study on behalf of EECDRG

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Abbreviations

D	day
DHC	dihydrochloride
DKG	German Contact Dermatitis Research Group (Deutsche Kontaktallergie-Gruppe)
ICDRG	International Contact Dermatitis Research Group
IVDK	German Information Network of Departments of Dermatology (Informationsverbund Dermatologischer Kliniken)
EECDRG	European Environmental and Contact Dermatitis Research Group
p-	para-
PPD	p-phenylenediamine
vol.	volume
wt	weight

Abstract

Background

The recommended routine patch test concentration for p-phenylenediamine (PPD) has been 1.0 % petrolatum since it was included in baseline set-up of patch test allergens. For over a decade it has been discussed if it is safe to routinely patch test PPD in this concentration due to the risk of patch test sensitization. Late appearing patch test reactions may reflect patch test sensitization, but may also be due to a low degree of pre-existing sensitization.

Objectives

The aim of this study was to follow the positive patch test reactions to PPD and its salt PPD-dihydrochloride (PPD-DHC) in serial dilution in order to characterize reaction patterns concerning time and dose in a cohort of individuals who were already PPD-sensitized.

Methods

Volunteers with previous reactions to PPD 1.0% were included in Barcelona, Gentofte, Odense and Malmö. They were patch tested with PPD and PPD-DHC in equimolar dilution series. Observation of the reactions on 7 follow-up visits during 28 days followed.

Results

Twenty-six test subjects completed the study of which 23/26 (88%) reacted to PPD 1.0%, whereas 69% reacted to 0.32%. Altogether, 42% and 27% reacted to the corresponding equimolar concentrations of PPD-DHC. Seventeen subjects (74%) were positive to 1.0% and 0.32% PPD on day 2. After day 7 no new reactions were observed to any concentration tested, either of PPD or of PPD-DHC. Eight (31%) of the test subjects who were previously PPD-positive and 5 (22%) of the subjects who were currently positive to 1.0% PPD were negative on testing with 0.32% PPD on days 2-7.

Conclusion

No late appearing reactions could be explained by PPD or of PPD-DHC as such or the dose. We could clearly see a risk of missing contact allergy when the dose was decreased.

Introduction

p-Phenylenediamine (PPD) is a potent contact sensitizer and a common constituent of permanent hair dyes. PPD's sensitizing capacity has been known for over 100 years. As early as in 1939 PPD was suggested for screening for hair dye allergy and was therefore included in one of the early baseline series for patch testing as 2% petrolatum (pet.) (1). In the International Contact Dermatitis Research Group (ICDRG) baseline series dated 1974 PPD was included as 1% pet (2). The recommended routine patch test concentration for PPD in order to establish that there is contact allergy in sensitized patients has been 1.0% pet. and PPD is currently still included in the European baseline series as 1.0% pet. (3).

For over a decade, it has been discussed whether PPD is a safe contact allergen for routine patch testing due to the risk of active sensitization, i.e. making non-sensitized patients sensitized by the patch test itself, and whether or not the dose used in the patch test should be reduced for the same reason (4-17). In the present study the aim was to investigate occurrence of late appearing reactions in already sensitized individuals where the explanation cannot be active sensitization.

When the patch test statistics of the German Information Network of Departments of Dermatology (IVDK) in the years 1995–2004 were analyzed 4% of 83,030 individuals tested were PPD-positive (8). In this material a high rate (44%) of PPD reactions without any known current relevance was found (8). Hillen et al performed prospective investigations in order to study late occurring reactions in routine patch testing with PPD 1% pet. (14). Readings were performed on D3, 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 1.9% (n=21) had positive reactions on D7 and beyond. Among the 21 patients with late reactions three had de novo reactions at D7 and the remaining 18 had positive reactions appearing at readings on D11–D37. Seven of the 21 patients were retested with PPD 1% and in 5 of 7 there was a positive reaction within 3 days on re-testing (14). Based on these result Hillen et al. estimate an active sensitization rate of around 1% in patients routinely patch tested with PPD 1.0% applied for 48h (14).

Based on this data removal of PPD at 1.0% from the baseline patch test series was recommended in Germany by DKG (German Contact Dermatitis Research Group), with the suggestion of only performing aimed testing (14). To avoid active sensitization, patch testing was only recommended if there was a clear suspicion of possible allergic contact dermatitis due to PPD from the patient's medical history and distribution of eczema. Lower patch test concentrations of 0.30–0.35% PPD have been proposed (4, 14).

The delayed patch test reaction may also be due to other reasons than active sensitization, e.g. the chemical properties and skin permeation. The drawback of only performing aimed testing is that the patient's history may not disclose exposure to PPD. Apart from PPD being a substance for diagnosing contact allergy to hair dyes in general, it is known that several other

allergens can induce cross-reactions 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 1984–1988 it was included in the baseline series as its salt, PPD dihydrochloride (PPD-DHC) (2). On recommendation of the European Environmental and Contact Dermatitis Research Group (EECDRG) and ICDRG, this was abandoned in favor of going back to testing with PPD, since too many allergies were being missed (2).

The aim of this study was to follow the morphology of the 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 7 occasions during 28 days to detect any late appearing reactions which might be misinterpreted as signs of active sensitization.

Materials and methods

Chemicals
The following chemicals were used: PPD (>99%; Sigma-Aldrich, St Louis, MO, USA), PPD-DHC (>99%; Fischer Scientific, Bridgewater, NJ, USA), ethanol (>99,5%; CCS healthcare, Borlänge, Sweden) and distilled water (Millipore; Q-guard 1, Molsheim, France).

Sample preparation
PPD and PPD-DHC were dissolved in ethanol/water 70:30 vol./vol. at the equimolar concentrations of 1.0% and 1.7% wt/vol., respectively. The pH of the PPD and PPD-DHC were 8 and 3, respectively. These stock solutions were further diluted in ethanol/water with the dilution factor √10 yielding 11 concentrations per chemical (table 1). Ethanol/water (70:30 vol./vol.) was chosen as vehicle, due to 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 one week.

Patch testing
The dilution series of PPD and PPD-DHC were applied on the backs of the volunteers in Finn chambers, diameter 8 mm (Epitest, Tuusula, Finland, now Smart Practice, Phoenix, Arizona, USA). A micro pipette was used to apply 15 µl of each test solution to the test chambers (18). Each person was patch tested with 11 doses of PPD and PPD-DHC respectively (table 1). Patch tests were removed after 2 days, D2.

Patch test reading
Patch tests were evaluated and scored after 2 days (minimum 30 minutes after patch test removal) followed by readings on D3 or 4, 7, 10, 14, 21 and 28. Patch test reading was

performed according to the criteria of the ICDRG (19). Patch tests were evaluated and scored after 2 days (a minimum of 30 min after patch test removal), followed by readings on D3/4, 7, 10, 14, 21, and 28. For each volunteer seven protocols were used, one for each patch test reading. The protocol consisted of two separate tables for PPD and PPD-DHC. A list for scoring was included, as well as 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 a help for the patch test readers to make the overall scoring. Based on the morphological scoring, each reader of a test made an overall evaluation with score +/++/+++/doubtful/irritant/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.

Study subjects

The inclusion criteria were an age of at least 18 years and a previous positive test reaction to PPD. In four study centers, dermatitis patients with previous positive reactions to PPD were identified from patch test data records, contacted, and invited to participate either by phone call or by letter. Patch tests were performed in 2011 and 2016 in Malmö, 2014 in Gentofte and Odense, and 2015 in Barcelona. Altogether, 34 study subjects were included (6 in Barcelona, 10 in Gentofte, 6 in Odense, and 12 in Malmö).

Ethics

The study was approved by the regional ethical boards in Sweden, Denmark and Spain. The study was conducted in accordance with the ethical standards specified in the Declaration of Helsinki.

Results

Of the 34 study subjects 26 completed the study, i.e. they were patch tested with dilution series of PPD and PPD-DHC and the test area was read 7 times according to the protocol as presented in Table 2.

23/26 (88%) reacted to PPD at 1.0%, whereas 69% reacted to 0.32%; 42% and 27% 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 its salt (PPD-DHC). 8 (31%) of the previously PPD-positive test subjects and 5 (22%) of those who were currently positive to PPD at 1.0% were negative on testing with 0.32% PPD on days 2–7, whereas the same number (n = 17) were positive to PPD at 1.0% and 0.32% on day 2. Reactions that did not appear until day 7 occurred in 4 of 26 study subjects (15%; volunteers 17, 19, 22, and 23). This pattern, 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. The total number of positive reactions over time is shown for the 3 highest concentrations of PPD tested in Figure 1.

Generally, positive reactions appeared between days 2 and 7 and then decreased in intensity. All these had disappeared by day 28.

15 study subjects had positive reactions to PPD-DHC at 1.7%. Reactions that did not appear until day 7 occurred in 2/26 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 3 highest concentrations of PPD-DHC tested. Generally speaking, positive reactions appeared on days 2 to 7, then decreased in intensity. All these had disappeared by day 28.

The test areas that were treated with corticosteroid cream due to extreme reactions, which occurred in 6 study subjects on D2 or D4, were not read further i.e. from test reading D4 or D7. When we analyzed the number of reactions over time, these treated areas were counted as positive up to D14 and negative from D21, in accordance with the trend observed for untreated +++ reactions.

Discussion

Active sensitization, synonymous to patch test sensitization, a possible adverse effect of patch testing, is sensitization caused by the patch test procedure. Patch test sensitization is usually indicated if a positive patch test reaction develops after D7, and often after D10-14 and beyond. If a subsequent re-test with the same preparation gives a positive reaction within 7 days, the initial late-appearing positive reaction is due to suspected active sensitization (20, 21). In Malmö re-testing is also performed with dilution series of the allergen based on a paper published by Bruze in 1984 (22). A retest with a dilution series will often help to discriminate between patch test sensitization and a late appearing positive patch test. A late patch test reaction is thus not always due to patch test sensitization (23-25).

Causes of variation in reaction time and sensitivity to an allergen may be the chemical properties, skin permeation, inter-individual variation in the expression of involved metabolic enzymes and anti-inflammatory influence from the allergen. Repeated topical exposure may affect the level of sensitivity. Increased sensitivity in an experimental study done by Mose et.al. has been reported between first and second challenge with diphenylcyclopropenone (DPCP) in human volunteers sensitized with DPCP suggestive of a booster effect between first and second patch test and thereafter a plateau level of reactivity following the next 4-5 challenges with 1 month interval (26). Late patch test reactions have been observed for some contact allergens. This has been described for corticosteroids, gold, acrylate, isocyanates and Compositae allergens (23, 25, 27-29).

It has been demonstrated 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 one of 10 patients the lower test doses elicited positive reactions on D10 and D21 while reactions to the highest concentrations developed within 7 days (23). Thus,

there is a possibility when patch testing 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 give 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 the present study to investigate reactions to PPD dilution series over time and see if we could detect any late reaction first appearing at 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 study subjects. None of the 26 study subjects had late-appearing reactions after D7 to PPD (Table 2). We did not see any late-appearing reactions to PPD-DHC either, although due 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 3 volunteers with new reactions at reading D7 (for PPD in subject no.20 and for PPD-DHC in subjects no. 9 and 11). When comparing D2 and D4, we saw new reactions developing at D4, to lower concentrations in 8 cases (for PPD in subjects no. 2, 5, 6, 7, 15 and, for PPD-DHC in no. 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)" (30). If reactions appearing on D7 are considered as being late reactions, 12% (n=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 reported a rate of 1.5% (n=21/1,428) 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 material, which is still a high figure for 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 at 1.0%, with subsequent recommendation to lowering the patch test concentration to 0.3%, to reduce the risk of active sensitization (14). Others have questioned these findings meaning that the risk for active sensitization has been overestimated and have promoted the keeping of PPD 1% for routine patch testing to avoid missing contact allergy to PPD (5, 13, 16, 31).

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 in 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 in different patch test centers. 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 the present study would miss 10/26 (38%) of the previously PPD-positive individuals, or 7/23 (30%) of the currently positive test subjects. In this study, 4/23 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. (4). Also, removal of PPD from the baseline series has been suggested with the recommendation to only test with it when there is a direct suspicion of contact allergy to PPD, in order to avoid patch test sensitization (17). In the present study, 23/26 study subjects reacted to PPD at 1.0%, whereas 18/26 reacted to 0.32% PPD. Lowering the routine patch test concentration to 0.3% might have missed diagnosis of contact allergy to PPD in 5 study subjects (22%) among those who showed reactions, and in 8 (31%) among subjects who were previously found to be PPD-positive. A defined dose per unit area rather than concentration is crucial when evaluating the risk of patch test sensitization. The elicitation and sensitization capacity is not only dependent on the concentration, but chiefly on the dose per unit area of allergen delivered to the skin and exposure time (3, 32). In a recent study good agreement was shown to PPD patch tests of equivalent doses of 1.0% and 0.3% pet. preparations (33). If dosed correctly PPD 1% may be safe to patch test.

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 looks more like an irritancy 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 reacted with late-appearing reactions to any concentration tested, either with the base or the salt. Even though late reactions to PPD that are due to causes other than patch test sensitization might occur, they appear to be infrequent. Chaudry et al. conducted a test reading once sometime between day 7 and day 14 and found no new positive reactions to PPD (34). Aalto-Korte et al. reported late reactions in a material of 826 patients tested with PPD, on day 10–14, in 6 cases (35), who then reacted early on re-test. The risk of patch test sensitization must be considered when routinely patch testing with 1.0% PPD. On the other hand, the risk of missed contact allergy must be considered, if reducing the test concentration of PPD or excluding PPD altogether from routine patch test. In this study, testing with 0.32% PPD at the most would have resulted in false negative reactions in 8 (31%) of the test subjects who were previously positive to PPD.

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

Figures and tables

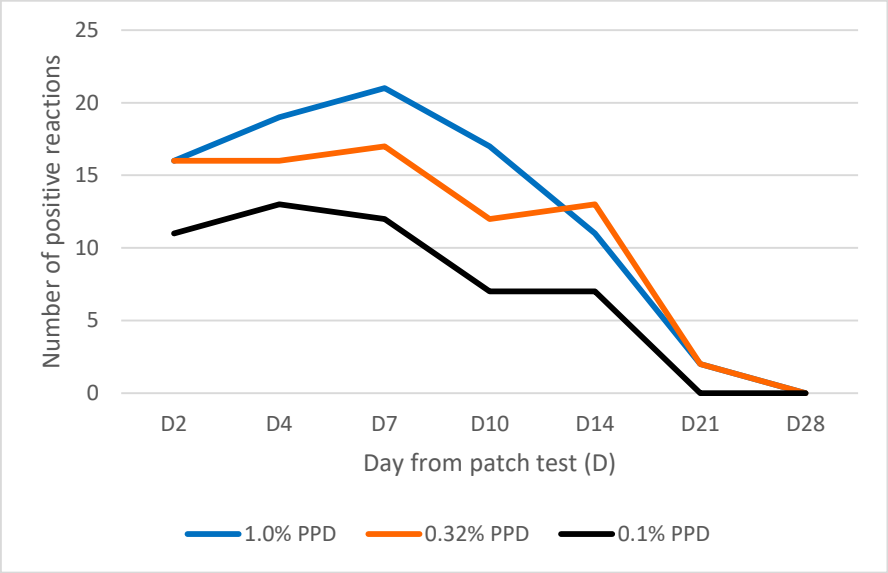

Figure 1. Number of positive reactions to p-phenylenediamine (PPD) over time.

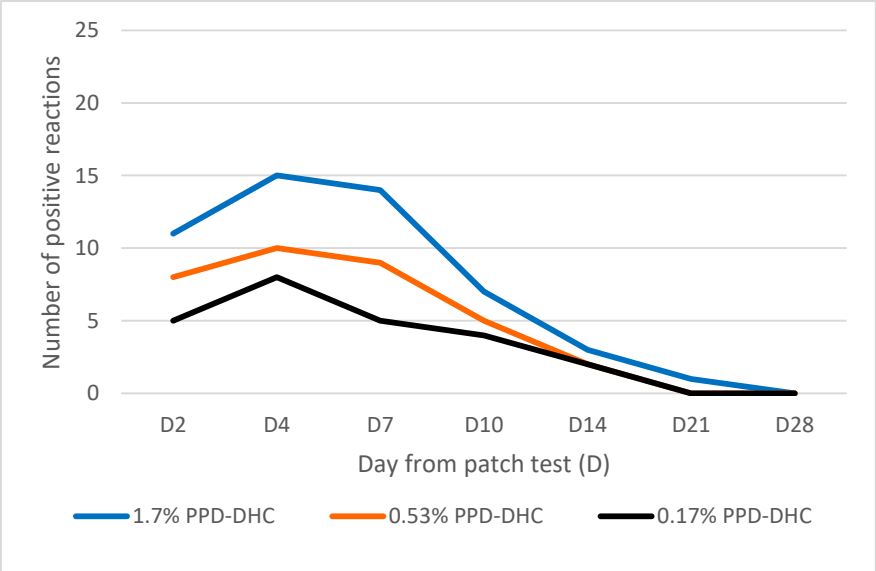

Figure 2. Number of positive reactions to p-phenylenediamine dihydrochloride (PPD-DHC) over time.

Table 1. The eleven equimolar concentrations of p-phenylenediamine and p-phenylenediamine dihydrochloride that were patch tested.
PPD, p-phenylenediamine; PPD-DHC, p-phenylenediamine dihydrochloride; v, volume; w, weight.

PPD % w/v	PPD-DHC % w/v
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

Table 2 Part 1/3. Test results for 26 volunteers tested with p-phenylenediamine (PPD) and p-phenylenediamine dihydrochloride (PPD-DHC): PPD 1.0% and 0.32%; PPD-DHC.1.7% and 0.53%. D4-readings were performed on either D3 or D4. Volunteer 19 was read on D9 instead of D10 and on D29 instead of D28.

Pat.	**PPD** 1.0 %							**PPD-DHC** 1.7 %							**PPD** 0.32 %							**PPD-DHC** 0.53 %						
	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	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	C
11	++	++	++	+	+	-	-	++	++	++	+	-	+	-	++	++	++	+	+	-	-	-	-	+	-	-	-	-
12	+++	C	C	C	C	C	C	+++	+++	+	-	-	-	-	+++	C	C	C	C	C	C	++	+++	+	-	-	-	-
13	+	+++	++	+	+	IR	-	-	++	+	-	-	-	-	+	++	+	-	-	IR	-	-	-	-	-	-	-	-
14	++	+++	+++	+	IR	-	-	-	++	+	-	IR	-	-	++	++	++	-	IR	-	-	-	-	-	-	-	-	-
15	+	++	-	-	-	-	-	-	-	-	-	-	-	-	+	++	-	-	-	-	-	-	-	-	-	-	-	-
16	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	-	++	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
19	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	+	++	++	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
21	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	-	-	++	++	-	-	-	-	-	-	-	-	-	-	+	++	++	++	+	+	-	-	-	-	-	-	-	-
23	-	-	++	+	-	-	-	-	-	-	-	-	-	-	-	-	++	+	+	-	-	-	-	-	-	-	-	-
24-26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PPD, p-phenylenediamine; PPD-DHC, p-phenylenediamine dihydrochloride; NT, not tested; +, weak positive; ++, strong positive; +++, extreme reaction; IR, irritant reaction; C, cortisone cream treatment.

Table 2 continued. Part 2/3. Test results for 26 volunteers tested with p-phenylenediamine (PPD) and p-phenylenediamine dihydrochloride (PPD-DHC): PPD 0.1% and 0.032%; PPD-DHC.0.17% and 0.053%. D4-readings were performed on either D3 or D4. Volunteer 19 was read on D9 instead of D10 and on D29 instead of D28.

Pat.	**PPD** 0.1 %							**PPD-DHC** 0.17 %							**PPD** 0.032 %							**PPD-DHC** 0.053 %						
	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	-	-	-	-	-	-	-	-	++	++	+	-	IR	-	-	-	-	-	-	-	-
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	-	-	-	-	IR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	-	++	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16-26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PPD, p-phenylenediamine; PPD-DHC, p-phenylenediamine dihydrochloride; NT, not tested; +, weak positive; ++, strong positive; +++, extreme reaction; IR, irritant reaction; C, cortisone cream treatment.

Table 2 continued. Part 3/3. Test results for 26 volunteers tested with p-phenylenediamine (PPD) and p-phenylenediamine dihydrochloride (PPD-DHC): PPD 0.001% and 0.00032%; PPD-DHC 0.0017% and 0.00053%. D4-readings were performed on either D3 or D4. Volunteer 19 was read on D9 instead of D10 and on D29 instead of D28. Among the 3 lowest concentration tested for PPD and PPD-DHC not presented in this table volunteer 1 had + to 0.0001% and 0.000032% PPD.

Pat.	**PPD** 0.001 %							**PPD-DHC** 0.0017 %							**PPD** 0.00032 %							**PPD-DHC** 0.00053 %						
	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	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PPD, p-phenylenediamine; PPD-DHC, p-phenylenediamine dihydrochloride; NT, not tested; +, weak positive; ++, strong positive; +++, extreme reaction; IR, irritant reaction; C, cortisone cream treatment.

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