

Assessment of the elicitation response in subjects weakly sensitized to *p*-phenylenediamine

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Summary

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Conflicts of interest

C.G. is an employee of the Procter & Gamble Company. The hair dye ingredient studied in this paper is currently used in commercial products marketed by the Procter & Gamble Company. All authors participated actively in the study. L.M.P. and P.J.C. as study directors; B.B. and C.G. as experts in skin sensitization. The authors alone are responsible for the content and writing of the paper.

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Background A 30-min application of a hair dye product containing 2% *p*-phenylenediamine (PPD) to subjects diagnostically graded +, showed that 12 of 18 reacted; eight of 18 with a true + and four of 18 with a doubtful (?+) response, whereas six of 18 did not react at all. *In vitro* skin-binding experiments showed that for diagnostic patch test conditions the measured exposure level (MEL) is more than 10-fold higher than the MEL for hair dyeing conditions.

Objective To further analyse the limited elicitation response of the diagnostically + graded subjects to a PPD hair dye product, under standardized test conditions mimicking product usage, by varying exposure time and dose.

Methods A hair dye model formulation containing 2% PPD, applied for 30, 45 and 60 min and a diagnostic PPD TRUE test[®] were applied to assess elicitation responses to increasing PPD exposure levels. Grading was performed according to International Contact Dermatitis Research Group guidelines.

Results Six subjects were available for this follow-up study. One of six subjects responded with a + elicitation response to the hair dye model applied for 60 min. Four of the five remaining subjects elicited a + response to the PPD TRUE test[®] applied subsequently, while one of five responded doubtfully.

Conclusions Increasing the PPD exposure time twofold – resulting in a 5–6% increase of sensitivity of this hair dye model test – or further extending the exposure time 48-fold, was found sufficient to increase the MEL above the thresholds needed to elicit individuals with a + diagnostic PPD patch test who did not react to typical hair dye use conditions with a MEL of about 6.8 µg cm⁻². This analysis confirms that consideration of the MEL is a useful tool to better characterize thresholds of elicitation than consideration of the applied dose alone.

What's already known about this topic?

- Elicitation of individuals sensitized to *p*-phenylenediamine (PPD) is dose dependent: after application of a hair dye formulation containing 2% PPD for 30 min to 18 subjects weakly sensitized (+) to PPD, six (33%) did not react and four (22%) showed only a doubtful response.
- Experimentally derived measured exposure levels (MEL) to PPD showed that the MEL for this hair dye condition is 6.8 µg PPD cm⁻², whereas a diagnostic 1% PPD–petrolatum patch test for 48 h resulted in a MEL of 205 µg PPD cm⁻².

What does this study add?

- Increasing the MEL to $16 \mu\text{g PPD cm}^{-2}$ – by doubling the exposure time of a 2% PPD hair dye formulation to 60 min – showed a minor increase of 5–6% in elicitation responses of the nonresponders and doubtful responders from a previous study. Applying a PPD TRUE test[®] indicated that a further increase in MEL (estimated to be in the region of $45 \mu\text{g PPD cm}^{-2}$) is associated with a major increase (four of five) in positive elicitation responses.
- By combining elicitation data with measured PPD exposure levels (MEL), a range of exposure conditions could be established, within which responses can be elicited in a number of subjects weakly sensitized to PPD. This confirms that exposure dose and time determine the MEL and thus the threshold of the elicitation response.

P-phenylenediamine (PPD) is well recognized as one of the potent sensitizers responsible for allergic contact dermatitis occurring in relation to hair dyeing. In a typical oxidative hair dyeing procedure, a colour cream containing precursors [such as PPD or p-toluenediamine (PTD) and/or p-aminophenol] and different couplers (e.g. m-aminophenol and resorcinol) is mixed with an oxidative developer (hydrogen peroxide) prior to application to the scalp. In a previous study,¹ we applied such a hair dye mixture, serving as a model simulating hair dye use conditions, to individuals with a well-documented history of hair dye-related allergic contact dermatitis (ACD) and a positive diagnostic patch test to PPD. Briefly, a hair dye finished product model formulation (2% PPD in combination with two couplers mixed with the developer), was applied to the ventral forearm using a Van der Bend square patch test chamber (Van der Bend, Brielle, the Netherlands). After 30 min, the chamber was removed and the application site was rinsed with water and shampoo. All subjects formerly graded ++ or +++ by diagnostic patch testing, reacted positively, whereas out of 18 subjects diagnostically graded +, eight reacted with a + response, four showed a doubtful reaction and six did not react at all. As the elicitation response to PPD is known to be affected by dose and exposure time,^{2–4} the aim of the current study – which is a continuation of the previous study¹ – was to further analyse the limited elicitation response of the + graded subjects to PPD under standardized (hair dyeing) conditions, by varying exposure time and dose. Importantly, there is a difference between the dose applied and the dose becoming available for elicitation. The latter, reflecting the actual exposure, can be expressed as the measured exposure level (MEL). The MEL for hair dye usage conditions (30-min application of PPD containing oxidative hair dye, followed by rinsing with water and shampoo) is calculated as the sum of PPD concentrations on/in the skin and receptor fluid and it was derived from *in vitro* skin-binding experiments in our previous study.¹ It was shown that under hair dyeing conditions, there is a linear correlation between the exposure time and the MEL

for PPD for exposure times from 5 min to 1 h. It was also demonstrated that under diagnostic patch test conditions, the actual exposure to PPD is more than an order of magnitude higher than under simulated hair dye conditions.¹ This article comprises a detailed assessment of the elicitation response under differing exposure conditions, related to the corresponding exposure levels, in individuals who had a weak response to a patch test with PPD. In addition, an interview addressed the topics of temporary 'black henna tattoos' and the hairdressing profession, because of the very strong relationship with exposure to PPD.⁵ Furthermore, in order to evaluate PPD patch test results accurately, concomitant positive patch test reactions were recorded.

Material and methods

Subject selection

Our previous study ('hair dye model F') showed that on exposure to a hair dye model formulation containing 2% PPD, six of 18 subjects graded + in the diagnostic PPD

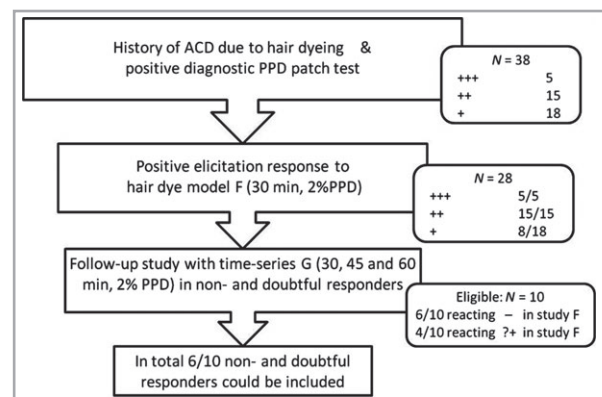


Fig 1. Flowchart showing the selection of subjects for the current time-series G, on the basis of their reaction to the previous hair dye model F study.¹ ACD, allergic contact dermatitis; PPD, p-phenylenediamine.

Table 1 Subject characteristics; all subjects had a history of symptomatic hair dye-related dermatitis, previously confirmed by + reaction to a diagnostic 1% PPD–petrolatum patch test. Additional information regarding the symptoms during the hair dye dermatitis and possible other sources of PPD exposure are shown

Subject	Sex, age (years) at first episode of hair dye dermatitis	Complaints, hair dye-related dermatitis	Have been a hairdresser?	Ever had a temporary 'henna tattoo'?	Current hair dye use	Diagnostic PTD 1% in pet. patch test result	Additional skin diseases
1	F, 43	Itch, erythema and infiltration of the scalp and fingers	No	No	Still dyeing; no complaints; no PPD or PTD in hair dye	+	Irritant hand eczema Sensitization: nickel
2	F, 28	Itch, erythema and infiltration of the scalp, forehead and ears	No	No	No	NT	Atopic eczema Sensitization: Balsam of Peru, fragrance mix
3	F, 36	Itch, erythema, infiltration and papules of scalp and neck	Yes, in the past	Yes, black; resulted in allergic reaction; occurred after hair dye dermatitis	No	?+	Atopic eczema Sensitization: Balsam of Peru, clioquinol
4	F, 61	Itch, burning sensation and infiltration of the scalp and forehead	No	No	Still dyeing; with PTD (0.3%)-containing hair dye; mild complaints	+	Sensitization: benzocaine, nickel, dibromodicyanobutane-phenoxylethanol, cocamidopropyl betaine
5	F, 20	Itch, erythema, infiltration, oozing vesicles and desquamation of scalp and both hands	Yes, stopped because of ACD	No	Still dyeing; with PTD (0.5–1%)-containing hair dyes; mild complaints	–	Sensitization: cobalt, ammonium persulphate, benzophenone-4 10%
6	F, 46	Itch, erythema and infiltration of the scalp, forehead, neck and ears; swollen eyelids	No	No	Still dyeing; with PTD (0.5–0.7%)-containing dye; without complaints	?+	Sensitization: fragrance mix, nickel

ACD, allergic contact dermatitis; PPD, p-phenylenediamine; PTD, p-toluenediamine; ?+, doubtful response; NT, not tested.

patch test did not elicit a response and four of 18 reacted doubtfully (+?). All subjects of this subpopulation of nonresponders and doubtful responders had a history of hair dye-related dermatitis; of these, four nonresponders and two doubtful responders were available for retesting the dose and time dependency of their elicitation responses in this current study (referred to as time-series G), which is a continuation of the previous hair dye model F study (Fig. 1). Information regarding the subject's history of hair dye dermatitis, regarding temporary 'black henna tattoo' or occupational (hairdresser) exposure and presence of concomitant skin diseases, was obtained by an interview (Table 1). Approval for retesting with time-series G and a diagnostic PPD patch test was obtained from the ethical committee of the University Medical Center Groningen.

Test substances

The hair dye ingredients for our time-series G were similar to the ingredients used in the former experiments.¹ In brief, the complete hair dye model consisted of a typical basic hair dye formula to which the dye precursors had been added. Four per cent PPD was added as the dye precursor together with the dye couplers 2-methylresorcinol (3.6%) and 2-methyl-5-hydroxy-ethylaminophenol (1.9%), which were chosen based on their very low sensitizing potency.^{6,7} Prior to application, the dye formulation (containing 4% PPD) was mixed with a developer solution (containing 6% hydrogen) in a 1 : 1 ratio, to form a 2% PPD-containing hair dye

model, equal to the model used in the previous study. An amount of 150 mg hair dye test solution (corresponding to 3000 µg PPD cm⁻²) was applied on a Van der Bend square patch test chamber and left on the volar forearms of the subjects for 30, 45 and 60 min. A control patch, designated as 0% (PPD) and consisting of the basic formula (without PPD and couplers) mixed 1 : 1 with the developer, was applied adjacent to the 2% patch, for each time frame (Fig. 2). After 30, 45 and 60 min, respectively, the patch test chambers were removed, the surface excess was wiped once with a tissue and subsequently the skin was washed with water and shampoo to mimic the use conditions of a normal hair dyeing procedure. The 30-min 2% PPD-hair dye application is similar to the previous study and therefore considered a test of reproducibility. Patch test readings were performed at 2 days (D2) and 3 days (D3) post-application and graded according to the International Contact Dermatitis Research Group (ICDRG) criteria. If no, or a doubtful reaction occurred at the D3 reading, the reactions in these subjects were also read and graded 1 week post-application (D7).

Diagnostic patch test

In order to further increase the exposure level (MEL), subjects were additionally tested with a diagnostic PPD TRUE test[®] (Mekos, Hillerød, Denmark), applied on the back for 48 h. Patch test readings were performed as described above.

Results

Six females, with a well-documented history of hair dye-related contact dermatitis and a weakly positive (+) reaction to a former diagnostic patch test with PPD (1% PPD in petrolatum), with absent (*n* = 4) or doubtful reactivity (*n* = 2) to a hair dye product containing 2% PPD applied for 30 min (model F), were subjected to an analysis of the time and dose dependency of their elicitation response. For this purpose, a hair dye product, containing no (i.e. control) or 2% PPD, was applied for 30, 45 and 60 min, respectively (time-series G). None of the subjects reacted to the control. All subjects reacted negatively to the application of 2% PPD during 30 min (Table 2). Increasing the exposure time to 45 min yielded no positive elicitation responses (data not shown). After a further increase of the exposure time to 60 min, one individual (subject 2, Table 2) reacted positively with a + response at D3. When interviewed by telephone at D7, no symptoms were reported by the five nonresponding subjects. To further increase the exposure to PPD in the five nonresponders, a diagnostic PPD TRUE test[®] was performed; results of these patch tests (readings at D2, D3 and D7 when no conclusive reading at D3) are also shown in Table 2. Because subject 2 had already reacted to the time-series, she was excluded from this retesting. Thus, five subjects were tested with this dose, and in four of them the original diagnostic PPD patch test grading (+) could be confirmed, while one subject (no. 6) was graded as doubtful.

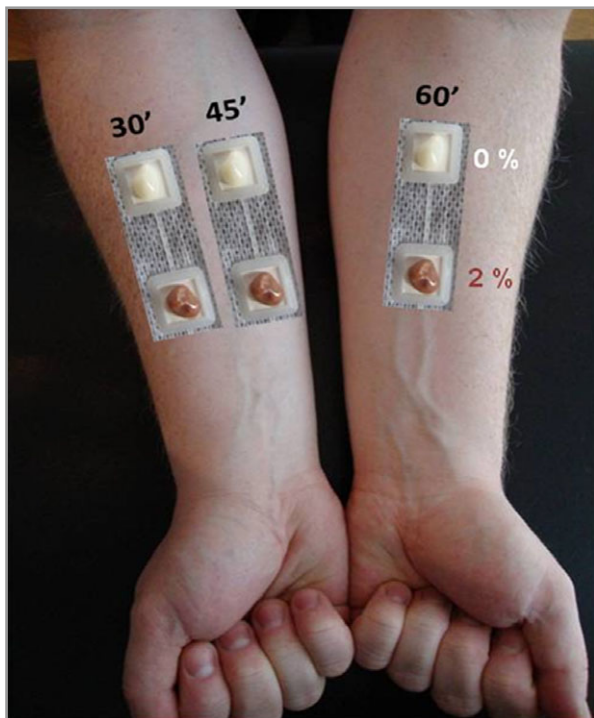


Fig 2. Schematic representation of the application of time-series G, showing the different dose and exposure time.

Table 2 Patch test grading results of subjects with a positive hair dye dermatitis history and a + response to a diagnostic 1% PPD–petrolatum patch test, but who failed to react to a 2% PPD hair dye model. Results of the original and confirmatory diagnostic patch tests, the previous hair dye model F and the current time-series G are displayed.

Application time PPD concentration (applied dose, $\mu\text{g cm}^{-2}$)		Diagnostic PPD patch test (1% PPD in petrolatum)	Hair dye model G			Diagnostic PPD patch test (TRUE test [®])
		48 h	30 min	60 min	48 h	
			2% ^a (3000)			
Subject	Reading day	1% (400)	F	G	2% (3000)	NS (90)
1	D2	—	—	—	—	?+
	D3	+	—	—	—	+
	D7			—	—	
2	D2	?+	—	—	—	
	D3	+	—	—	+	Not tested
	D7			—		
3	D2	—	—	—	—	?+
	D3	+	?+	—	—	+
	D7			—	—	
4	D2	—	—	—	—	?+
	D3	+	?+	—	—	+
	D7			—	—	
5	D2	—	—	—	—	?+
	D3	+	—	—	—	+
	D7			—	—	
6	D2	—	—	—	—	?+
	D3	+	—	—	—	?+
	D7			—	—	?+

^aThe experimental conditions of the F-column (results of the previous hair dye model F; Goebel *et al.*, 2010)¹ were equal (i.e. 2% PPD in hair dye formulation, 30-min application) to the G-column (results of the time-concentration series G, 30-min application). NS, not specified; PPD, p-phenylenediamine.

The previous relevance of the subject's hair dye-related dermatitis could be established by the interview. The interview revealed that subject 3 has also had a temporary 'henna tattoo' resulting in an allergic reaction. This reaction occurred after the hair dye-related contact dermatitis had taken place. Both subjects 3 and 5 are former hairdressers and subject 5 actually had to leave her profession due to eczematous complaints. Subjects 1 and 4 also reacted positively to PTD (1% in petrolatum), whereas subject 5 had a negative response, while subjects 3 and 6 reacted doubtfully to the PTD patch test. Next to the presence of irritant and atopic eczema, concomitant patch test reactions were found. Among them were some allergens known to cross-react with PPD or PTD (benzocaine and disperse-orange 3) or to be present in hair dye-related products or environments (fragrance mix, cocamidopropyl betaine, ammonium persulphate and nickel) (Table 1).

Discussion

We previously demonstrated that application of a hair dye product containing 2% PPD elicits a positive response in 73% of PPD diagnostic patch test-positive individuals (84% when any response, also not fulfilling the criteria for a true positive reaction, is taken into account).¹ By investigating how hair

dye use conditions compare with the conditions of diagnostic patch testing, we found that the actual exposure level (MEL) estimated after removal of the surface excess is more than an order of magnitude lower ($\text{MEL } 6.8 \mu\text{g cm}^{-2}$) than under diagnostic PPD patch testing ($205 \mu\text{g cm}^{-2}$), although sufficient to elicit a response in the majority of PPD patch test-positive individuals with a clear history of hair dye-related contact allergy. However, among 18 subjects diagnostically graded +, 12 reacted (eight with grade + and four doubtful) and six did not react at all to the MEL of $6.8 \mu\text{g cm}^{-2}$.¹ These results suggest that for some individuals the previous + patch test result was of no current relevance with respect to the hair dye elicitation model chosen and that the MEL during simulated hair dyeing conditions was presumably below the threshold of elicitation in these individuals. Here, we first aimed to investigate the reproducibility of our earlier results in the abovementioned four doubtful and the six nonreacting subjects to the application of the same hair-dye formulation. Finally, six of them (four negative and two doubtful responders) were available for this reinvestigation.

Using the same hair-dye formulation with 2% PPD applied for 30 min, we found that all six did not respond; even the previously doubtful reactors ($n = 2$) did not show a response. This limited reproducibility was in line with our expectations

in this apparently low-sensitized group of individuals, and is in line with typical findings for weak responders.^{8,9} Thus, we have shown that the PPD exposure becoming available from a 2% PPD-containing hair-dye formulation within 30 min (characterized by a MEL of $6.8 \mu\text{g cm}^{-2}$) is too low to elicit a reliable reaction in weakly PPD-sensitized subjects.

Consequently, we assumed that their elicitation threshold is above $6.8 \mu\text{g cm}^{-2}$ and addressed the question of whether increasing the MEL elicits a skin reaction in these subjects. Increasing the exposure time for the 2% PPD hair dye formulation to 60 min is expected to roughly double the MEL from 6.8 to $16 \mu\text{g cm}^{-2}$, based on the knowledge from our previous study showing that increases in exposure time induce a linear increase of the MEL.¹ Interestingly, doubling the exposure time did not substantially alter our findings. Only one of the six sensitized subjects responded while the other five individuals did not react, indicating that for five of these six subjects the elicitation threshold can be considered to be above $16 \mu\text{g cm}^{-2}$. In summary, taking the figures of our previous study¹ into account, the sensitivity of this hair dye model test increases by 5–6% if the exposure time is increased to 60 min; from 44% (eight of 18) to 50% (nine of 18) for weakly sensitized subjects and from 67% (12/18) to 72% (13/18) when doubtful responders are also included.

In order to further increase the MEL, we applied PPD using the 48-h diagnostic TRUE test[®] (equivalent to an applied amount of $90 \mu\text{g PPD cm}^{-2}$)¹⁰ to the five nonresponders. Based on the findings from our previous study¹ – which considered the MEL under patch test conditions to be approximately 50% of the dose applied – we extrapolated the MEL from the 1% PPD–petrolatum patch test to the PPD TRUE test[®]. Consequently, we assumed that the applied dose of $90 \mu\text{g PPD cm}^{-2}$ can be considered equivalent to a MEL of about $45 \mu\text{g cm}^{-2}$, which presumably is well above a MEL of $16 \mu\text{g cm}^{-2}$ and below the MEL from a petrolatum-based diagnostic patch test ($205 \mu\text{g cm}^{-2}$).¹ The TRUE test[®] exposure elicited a response in four out of these five subjects (Fig. 3). This reconfirms that exposure dose and time have a major impact on the MEL and thus on the elicitation response together with the degree of sensitization. Secondly, we learned that among grade + patch test cases with a previous history of hair dye contact dermatitis some individuals (six of 18) do not respond to a MEL occurring under hair dyeing conditions. However, it should be kept in mind that according to the ICDRG guidelines a + reaction covers a spectrum of morphological signs (ranging from just erythema and infiltration to possible papules) which implies that within a group of + responders, differences in elicitation thresholds are expected.

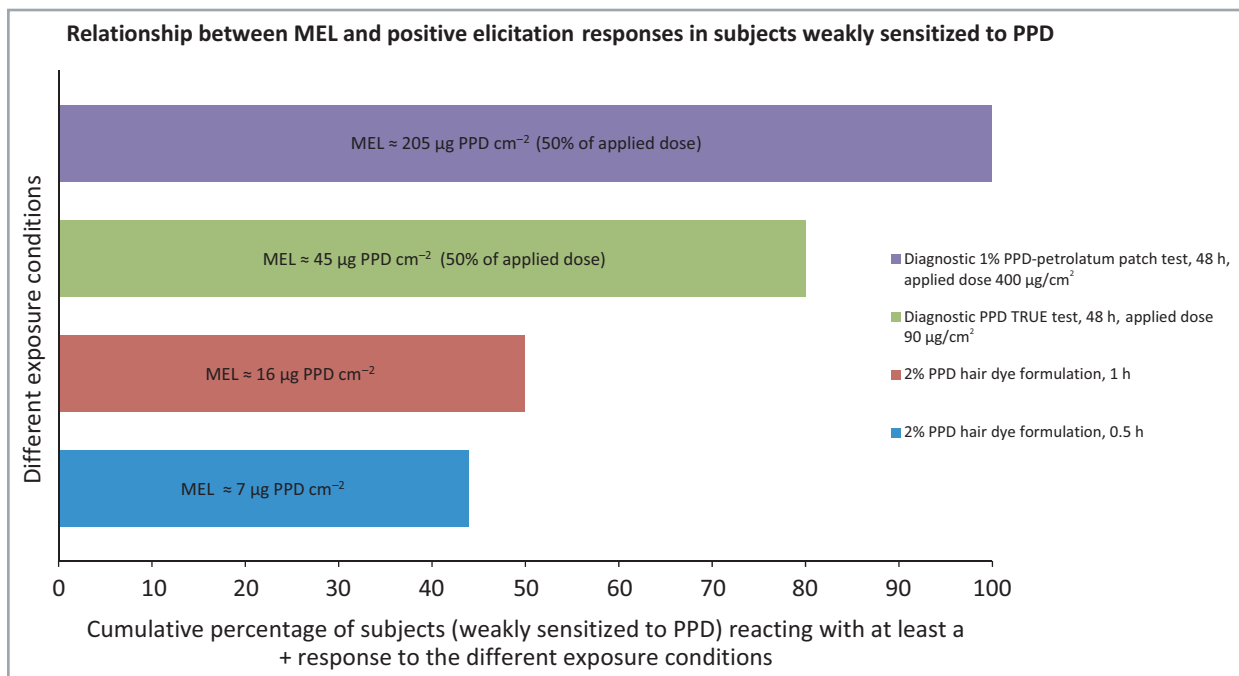


Fig 3. The 18 of 18 subjects weakly sensitized to PPD from the former hair dye model study F and subsequently the six subjects included in the current study all reacted to the 1% PPD–petrolatum patch test (applied PPD dose $400 \mu\text{g cm}^{-2}$, exposure time 48 h, corresponding MEL $\approx 205 \mu\text{g PPD cm}^{-2}$; 100% reaction rate). Applying the PPD TRUE test[®] (applied PPD dose $90 \mu\text{g cm}^{-2}$, exposure time 48 h) with a considerably lower MEL (presumably between 16 and $205 \mu\text{g PPD cm}^{-2}$ and estimated at $\approx 45 \mu\text{g PPD cm}^{-2}$ on the basis of Goebel *et al.*¹) resulted in four of five subjects (80%) reacting with a positive response. Considering the total population of weakly sensitized subjects (current and previous study), it is shown that eight of 18 subjects (44%) reacted with a truly positive (i.e. at least +, no doubtful) elicitation response to the 2% PPD hair dye model applied for 30 min (MEL $\approx 7 \mu\text{g PPD cm}^{-2}$). Doubling the exposure time of the hair dye model to 60 min (MEL $\approx 16 \mu\text{g PPD cm}^{-2}$) yielded one additional subject with a positive elicitation response, corresponding to nine of 18 or 50% of the subjects. PPD, p-phenylenediamine; MEL, measured exposure level.

Although the rate of nonresponders in our paper is somewhat lower, it is comparable with findings reported by Jowsey *et al.*¹¹ By applying a hair dye product containing a four fold lower PPD dose (0.5%) compared with the application of 2% as used by us, they found that none of their PPD-allergic individuals with a + diagnostic patch test response developed an elicitation reaction following a 30-min exposure and only two of 15 to a product with an unknown higher PPD concentration. Similarly to our study, Sosted *et al.*⁴ studied the elicitation threshold in PPD-sensitized subjects by a diagnostic patch test (48 h exposure) application containing different PPD doses (1–10 000 ppm) using petrolatum as vehicle. Subsequently, the probability of a positive response by a given dose was calculated by using a logistic regression model. It was shown that the elicitation threshold for 10% of the patch-tested persons (ED_{10}) was 38 ppm PPD. According to our calculation, this would correspond to a MEL of approximately $0.8 \mu\text{g PPD cm}^{-2}$. For the ED_{50} (330 ppm PPD) and ED_{90} (2800 ppm PPD), these values would correspond to a MEL of approximately 7 and $57 \mu\text{g PPD cm}^{-2}$, respectively. When comparing these derived values with our results as presented in Figure 3, it can be shown that the elicitation thresholds in our subjects are somewhat higher than those from Sosted *et al.* but clearly in the same order of magnitude: when we estimate the MEL for 50% of the subjects reacting with a positive elicitation response, this would be approximately $16 \mu\text{g PPD cm}^{-2}$ in our study compared with $7 \mu\text{g PPD cm}^{-2}$ in Sosted's study. The observed differences might be explained by the fact that our population consisted solely of weakly sensitized subjects, whereas Sosted *et al.* did not define their group in terms of sensitization status; their group may have contained stronger sensitized subjects for whom the elicitation threshold is assumed to be lower than for the weakly sensitized subjects.^{11–14}

The questionnaire revealed that for all six subjects the initial sensitization to PPD was likely based on exposure to hair dye; either as a consumer or as a professional (hairdresser). Subject 1 is still able to dye her hair without complaints. It turned out that the nonoxidative mid-brown hair dye she uses currently contains no PPD or PTD and therefore might explain the absence of complaints after hair dyeing. Interestingly, three others have also re-started to dye their hair. Thus, whereas currently two of the six subjects absolutely renounce the use of hair dye, the remaining four are still dyeing their hair. However, this is a phenomenon more often observed in subjects with a + reaction to a diagnostic patch test, unlike the ++ and +++ reactors, who more often restrain themselves from continuing the use of hair dye.¹⁵ The hair dyes which are being used currently by subjects 4–6 contained no PPD, but instead contained PTD (estimated on head concentrations of 0.3–1%, Table 1, personal communication). The presence of PTD might explain the mild complaints experienced by subject 4; who was also graded positively to a diagnostic 1% PTD–petrolatum patch test. Apparently, the mild complaints after using PTD-containing hair dyes (up to 1% PTD on head concentration) experienced by subject 5 are not caused by PTD itself, as the PTD patch test showed no sensitization.

These complaints might be explained by irritancy rather than allergy or due to her sensitization and possible use of ammonium persulphate in bleaching the hair.

In summary, by combining our clinical elicitation responses with previous experimentally derived exposure levels (MEL), we were able to establish a range of exposure levels within which responses can be elicited in 5–6 weakly PPD-sensitized subjects. Our findings further confirm that consideration of the MEL is a useful tool to more precisely estimate thresholds of elicitation than consideration of the applied dose alone.

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