

Different concentrations and volumes of *p*-phenylenediamine in pet. (equivalent doses) are associated with similar patch test outcomes: a pilot study

Flemming Andersen¹, Carsten R. Hamann^{2,3} , Klaus E. Andersen¹, Dathan Hamann⁴ , Eric Sager⁵ and Curtis Hamann⁶

¹Department of Dermatology and Allergy Centre, Odense University Hospital, Odense 5000, Denmark, and Dermatological Investigations Scandinavia, Department of Clinical Research, University of Southern Denmark, Odense 5230, Denmark, ²Department of Dermatology and Allergy, Herlev and Gentofte Hospital, University of Copenhagen, Hellerup 2900, Denmark, ³School of Health and Medical Science, Graduate Programme in Public Health and Epidemiology, University of Copenhagen, Copenhagen 1165, Denmark, ⁴Division of Dermatology, Department of Medicine, The Ohio State University, Columbus, OH 43230, USA, ⁵Loma Linda University School of Medicine, Loma Linda, CA 92350, USA and ⁶Contact Dermatitis Institute, Phoenix, AZ 85008, USA

doi:10.1111/cod.12951

Summary

Background. Concern about causing active sensitization when patch testing is performed with *p*-phenylenediamine (PPD) 1% pet. has led to a recommendation to use PPD 0.3% pet. as a potentially safer preparation. However, the dose per area of allergen delivered, and hence the risk of active sensitization, depend on the amount dispensed into the patch test chamber, which can vary widely.

Objective. To evaluate whether patch testing with equivalent doses of different concentrations of PPD in pet. is associated with similar outcomes.

Methods. Seventeen known PPD-sensitive subjects were patch tested with different volumes and concentrations of PPD in pet. that deliver the same allergen dose per unit area (6 mg of PPD 1% pet. and 20 mg of PPD 0.3% pet. in Finn Chambers[®], both equivalent to ~0.09 mg/cm²).

Results. Eleven patients (65%) had positive reactions to both doses; 4 patients (24%) had negative results [percentage agreement of 88% (15/17)]. One patient each had a positive reaction to only one dose.

Conclusions. The 88% concordance suggests that dose per unit area is more important in determining reactions to allergens than the excipient volume dispensed. Patch testing with a smaller volume of 1% PPD may be a reasonable alternative to testing with 20 mg of 0.3% PPD.

Key words: active sensitization; patch testing; PPD; *p*-phenylenediamine.

The blue–black aniline dye *p*-phenylenediamine (PPD) is an undisputed contact allergen. As early as 1961, the

Consumer Product Safety Commission identified PPD as one of only five chemicals that met the statutory definition of a ‘strong sensitizer’ in the United States (1). Although the primary route of sensitization to PPD is the use of hair dyes, a new pattern of exposure – temporary henna tattoos formulated with high levels of PPD – helped to earn the allergen its designation by the American Contact Dermatitis Society as the 2006 Contact Allergen of the Year (2). Other potential sources of exposure to PPD and its derivatives include textile dyes, photographic developing agents, printing inks, and black rubber products. With global median prevalences of sensitization among clinical patch test patients ranging from 2% to > 6%, PPD

Correspondence: Curtis Hamann, Contact Dermatitis Institute, 3400 E. McDowell Road, Phoenix, AZ 85008, USA. Email: hamann@smartpractice.com

Conflict of Interests: Dr Curt Hamann is CEO and owner of SmartPractice (Phoenix, AZ, USA), a company that produces and sells patch testing materials, and Medical Director of the Contact Dermatitis Institute; Drs Carsten R. Hamann and Dathan Hamann are his sons. Dr Klaus E. Andersen is the Medical Advisor for SmartPractice Denmark, which manufactures the patch test product, TRUE Test[®]. Dr Flemming Andersen and Mr Eric Sager have no relevant conflicts of interest to declare.

Accepted for publication 3 December 2017

was justifiably included in all major baseline series until recently (3).

In 2005, concerns about the risks of active sensitization from patch testing with 1% PPD caused the German Contact Dermatitis Research Group [Deutsche Kontaktallergie-Gruppe (DKG)] to remove this allergen from their baseline series. Their action was in response to a study by Hillen et al. that found that patch testing with PPD 1% pet. elicited late reactions [on day (D) 7 or beyond] in 1.5% of patients tested (4). This finding was interpreted to mean that patch testing with PPD 1% pet. might be actively sensitizing patients. The DKG then conducted a study to determine the appropriateness of patch testing with a lower concentration of PPD in pet., and found 0.35% to be both a safe and an effective dose, an outcome that was echoed in a later study using PPD 0.3% pet. (5).

On the basis of these studies, joint members of the Information Network of Departments of Dermatology and the DKG have recommended replacing PPD 1% pet. in the German baseline series with PPD 0.3% pet. (5). It would seem logical for manufacturers of contact allergens to offer PPD in pet. at this new, lower recommended concentration. However, the regulatory requirements associated with producing and licensing allergens, even a new concentration of a previously approved allergen such as PPD, are time-consuming. Consequently, a PPD 0.3% pet. formulation is not yet licensed, effectively eliminating the possibility of patch testing with this important allergen in Germany unless PPD sensitization is strongly suspected. We therefore investigated the efficacy of patch testing with a smaller amount of PPD 1% pet. that produced a dose equivalent to that currently recommended by the DKG, assuming widely accepted guidelines for the amount of allergen dispensed. The study was aimed at determining whether a lower amount of the 1% concentration could be used as a simple alternative until a licensed PPD 0.3% pet. formulation becomes available.

Materials and Methods

This prospective, single-centre, single-blinded, randomized study was approved by the Research Ethics Committee for the Region of Southern Denmark (Den Videnskabetiske Komité for Region Svddanmark). Informed consent was obtained from all participants. All participants were aged ≥ 18 years and willing to comply with the required study visits and other study restrictions (e.g. protecting panels from excess moisture, and not applying topical treatments to the test area). Participants had a known sensitivity to PPD, defined as a past positive patch test reaction to the allergen on

TRUE Test[®] (SmartPractice, Phoenix, AZ, USA) within the previous 10 years.

All patients were patch tested with two volumes and concentrations of PPD in pet. weighed and dispensed with a positive-pressure micropipette (Gilson Microman[®]; Gilson, Villiers-le-Bel, France) into 8-mm polypropylene-coated Finn Chambers[®] on Scanpor[®] tape (SmartPractice): 6 mg of PPD 1% and 20 mg of PPD 0.3%, which effectively produce the same dose per unit area. Dose per unit area of these allergens dispensed in a Finn Chamber[®] was calculated as follows: (6)

amount of allergen dispensed (mg) $\times$ allergen concentration (%) / patch test chamber area (cm²) = dose (mg) / unit area (cm²).

We used the full diameter of the Finn Chamber[®] (9.5 mm), not just the 8-mm diameter of the inner chamber depression, for calculations, to account for spreading of the pet. to the edges of the chamber, which results in an effective area of 71 mm (6). When the dose per unit area for 20 mg of PPD 0.3% (20 mg $\times$ 0.003/71) and 6 mg of PPD 1% (6 mg $\times$ 0.01/71) are calculated, it is apparent that both doses equal ~ 0.09 mg/cm². Both PPD pet. formulations were compounded at the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Lund University, Malmö, Sweden.

The chambers were applied to the skin of the paraspinal region of the patient's upper back and worn for 48 h (until D2). The investigator and subjects were aware of the allergens and the patch test doses, but were unaware of the configuration of the allergens on the individual test panels. The patch test sites were evaluated on D3 and D4 after patch application. Patch test reactions were graded according to ICDRG patch test guidelines (7). Subjects were instructed to contact the investigator if they observed the development of a late response after the final evaluation at D4. The frequency of each type of reaction was recorded for both formulations of PPD. Concordance between the two formulations was measured with Cohen's kappa coefficient, and 95% confidence intervals (CIs) for binomial proportions were estimated with the Clopper–Pearson method. Statistical analysis was performed with Excel software version 13.39 (Microsoft, Redmond, WA, USA).

Results

Seventeen subjects (15 females and 2 males) participated in this study. Their mean age was 49 years [standard deviation (SD): 4 years]. The subjects' historical patch tests had been conducted a mean of 6 years earlier (SD: 4 years). When the patches were removed on D2,

Table 1. Patch test reactions of 17 patients to 1% *p*-phenylenediamine (PPD) and 0.3% PPD formulations in pet. on day 3 and day 4, and to prior TRUE Test®

Skin reaction	1% PPD		0.3% PPD		Prior TRUE Test®
	Day 3, n (%)	Day 4, n (%)	Day 3, n (%)	Day 4, n (%)	n (%)
?	5 (30)	3 (18)	4 (24)	3 (18)	0
Negative	2 (12)	2 (12)	2 (12)	2 (12)	0
+	1 (6)	3 (18)	3 (18)	4 (24)	8 (47)
++	8 (47)	8 (47)	6 (35)	6 (35)	9 (53)
+++	1 (6)	1 (6)	2 (12)	2 (12)	0
Total positive reactions	10 (58.8)	12 (70.6)	11 (64.7)	12 (70.6)	17 (100)

Table 2. Agreement between patch test reactions in 17 patients to *p*-phenylenediamine (PPD) 0.3% and 1% pet. formulations on day 3 or day 4

Reactions to 0.3% PPD		Reactions to 1% PPD					
		Not positive			Positive		
		Irritant	–	–/+	+	++	+++
Not positive	Irritant	0	0	1	0	0	0
	–	1	0	0	1	0	0
	–/+	0	1	1	0	0	0
Positive	+	0	1	0	2	1	0
	++	0	0	0	0	6	0
	+++	0	0	0	0	1	1
Concordance		Four concordant negatives and 11 concordant positives = 15 (88.2%, 95%CI: 63.6–98.5%)					

adhesion was excellent in all 17 cases. The skin reactions to the two formulations are shown in Table 1. Overall, 13 subjects had a positive reaction to at least one allergen formulation. Two subjects had a positive reaction to one formulation each. The overall agreement between the two formulations was 88.2% (95%CI: 63.6–98.5%; Table 2), and the appearances of concordant positive reactions to the two formulations were similar (Fig. 1).

For both formulations of PPD, 2 subjects had more intense reactions than their historical reactions, and 6 subjects had less intense reactions than their historical reactions. When measured with Cohen's kappa coefficient, the interdose agreement was good, with a coefficient of 0.72 (95%CI: 0.35–1.0). The percentage agreement between each volume of PPD and the historical skin reaction was 71%.

Discussion

Allergy to PPD is a substantial global problem associated with marked regional differences in prevalence. The PPD allergy prevalence is higher in North America (historically, ~6%) than in Europe (~4%). However, there are significant regional differences even within Europe, with higher prevalences being reported in southern European countries than in northern European countries

(3, 8). On the basis of 2013–2014 data from the North American Contact Dermatitis Group, the prevalence of positive reactions to PPD was even higher, at 7%, and has steadily increased since 2000 (9). In Asia, the prevalence of PPD sensitization is ~4.4%, but prevalences as high as ~14% have been reported (3, 10). In contrast to the findings in most other countries, in Asia the prevalence is higher in males than in females (3). Although there are other avenues of sensitization, hair dyeing is an important and widespread source of exposure (8, 11, 12).

Given the global importance of this allergen, there is little argument that PPD belongs in the baseline series. The removal of PPD 1% pet. from the German baseline series because of safety concerns eliminates the ability to monitor contact sensitization to this recognized allergen in the context of a global environment in which PPD sensitization appears to be increasing.

Implications of dose

The length of exposure to an allergen and its concentration upon application are two important factors in eliciting patch test reactions. In their study, Hillen et al. noted that there were two alternatives to patch testing with PPD 1% pet. (4). One was to reduce patients'

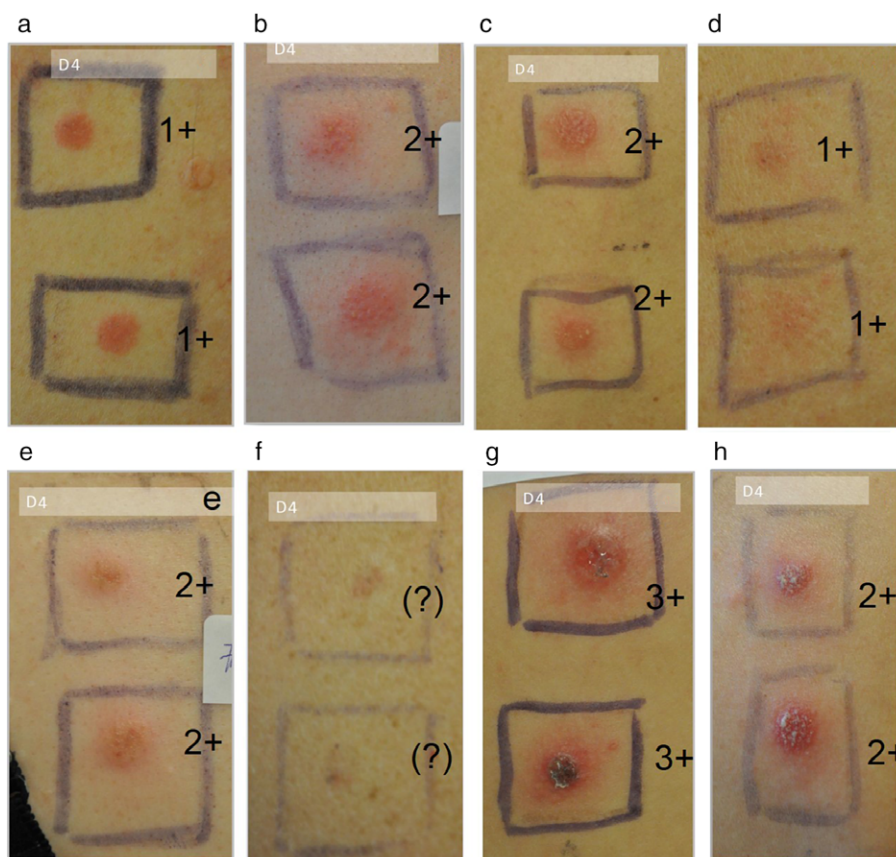


Fig. 1. Photographs of the test sites of 8 subjects on day 4 (D4) show the similarity in the appearance of concordant reactions to the two volumes of *p*-phenylenediamine tested. Neither investigator nor subject was aware of the configuration of the allergens on the test panels. Hence, the positions of the reactions to the 6-mg (0.3%) and 20-mg (1%) volumes vary across the top and bottom rows of each pair of reactions. Top row: (a) 1%, (b) 1%, (c) 0.3%, (d) 1%, (e) 1%, (f) 1%, (g) 0.3%, and (h) 0.3%.

exposure by limiting patch testing to 24 h (1 day) instead of the standard 2 days. This strategy is an option because the number of positive reactions elicited by PPD is directly proportional to exposure time (13, 14). However, using a 1-day exposure for only one allergen would probably create logistical problems in a typical patch test practice, and the 2-day exposure time is firmly established among the international patch test community. The second alternative was to reduce the concentration of PPD used for testing (4). Testing with too low a concentration of any allergen raises concerns about failing to identify patients who are, in fact, sensitized to the substance. Such a scenario can translate into sensitized patients not receiving the necessary treatment for their contact dermatitis – an outcome that is arguably more adverse than the risk of active sensitization. In the case of PPD, however, the DKG has determined that patch testing with PPD 0.3% pet. is safe in terms of both active sensitization and diagnostic reliability (5), and is thus a reasonable alternative to testing with PPD 1% pet.

An important point missing from this discussion – and that is too often overlooked in patch testing in general – is a precise definition of dose. There is a tendency for dose

to be reported as concentration alone (percentage). However, dose is determined by both the concentration and the amount of allergen dispensed in a defined space (i.e. chamber). To be precise and accurate, ‘test concentrations should preferably be expressed as weight per area, e.g., milligrams or micrograms per square centimeter’ (15). The distinction is not academic; it has important clinical implications, because dose per unit area is the critical factor for both sensitization and elicitation of contact allergy (16, 17).

Consequently, describing dose in terms of concentration alone is accurate only when the amount or volume of allergen dispensed is uniform across time and subjects while all other technique-related parameters are held constant (18, 19). Such uniformity, however, is rare in daily practice. Even in experienced patch test centres, intraindividual and interindividual differences in the amount of pet. allergen dispensed can vary by a factor or two or more (18). In general, 15 µl is widely accepted as the standard for an allergen in a liquid excipient dispensed into a Finn Chamber[®], whereas 20 mg has been proposed for pet.-based allergens (15, 20, 21). Recommendations for amount, however, have also been based on the length

of the ribbon of pet. allergen dispensed into the chamber (5 mm), presumably as a more easily reproducible visual approximation of 20 mg (7, 22). The optimal amount of allergen may also depend on the type of patch test system used (6).

Relying on concentration as an abbreviated descriptor of dose may obscure the point that different volumes and concentrations of PPD (or any allergen) can deliver the same dose per unit area. As a proof of concept, we compared the reactions of patients with known sensitivity to PPD who were patch tested with 6 mg of PPD 1% and 20 mg of 0.3% PPD in pet., which both deliver $\sim 0.09 \text{ mg/cm}^2$. The latter was selected to correspond to what would most likely be considered a standard amount (20 mg) of the PPD concentration recommended by the DKG (0.3%). The concordance of 88%, which indicates that the efficacy of the two 'doses' was similar, is consistent with the literature (23). As a potential short-term solution to the absence of a licensed PPD 0.3% pet. formulation, patch testing could be conducted with a smaller amount (6 mg) of the commercially available concentration of PPD 1% pet. rather than with the amount widely accepted as standard (20 mg).

There are potential caveats to, and criticisms of, this approach, however. Given the intraindividual and interindividual variation in preparing patch test substances, it would be prudent to measure 6 mg of PPD 1% pet. with a positive-pressure micropipette or a sensitive laboratory-grade scale to avoid overdispensing of the allergen. There could also be concerns that 6 mg is too small an amount to completely cover the test area delimited by a typical chamber. However, the relatively uniform appearance of the reactions of the subjects who reacted to both volumes of PPD suggests otherwise, and perhaps reflects the highly sensitizing nature of this allergen. Finally, the historical positive reactions in this study could have been obtained as long as 10 years before

the current study was conducted, and current relevance was not assessed.

Active sensitization

Subjects were not evaluated for late reactions, because active sensitization was outside the scope of the study. Nonetheless, concern about the risk of active sensitization motivated withdrawal of PPD 1% pet. from the German baseline series, and the topic warrants consideration. Active sensitization resulting from patch testing is typically considered to have occurred when a subject has a late positive patch test reaction to an allergen but shows a positive reaction within a few days when retested with the same substance (15). In general, reports of active sensitization to PPD are rare. Among 7619 consecutively patch tested dermatitis patients in Denmark, only 26 (0.3%) had late reactions (24). In only 1 case could the patient not exclude the possibility that her condition had worsened after the putative active sensitization, which her treating physicians considered to be unlikely. In a retrospective review of 298 patients patch tested at the Mayo Clinic, no new delayed reactions to PPD were seen (25). Studies of the same cohorts across time also suggest that a minimal risk of active sensitization is associated with patch testing with PPD in the general clinic population (26).

Conclusion

Our findings support the concept that patch testing with different volumes and concentrations of PPD that deliver the same dose per unit area elicits similar results. However, it is unclear whether this finding is generalizable to other allergens. Nevertheless, it may be reasonable to modify the dose delivered, especially of highly sensitizing allergens such as PPD, when different allergen concentrations are desired but unavailable.

References

- Pollitzer P M, Kameros A J. Hazardous substances and articles; administration and enforcement regulations: notice of proposed rulemaking; revisions to supplemental definition of 'strong' sensitizer. Consumer Product Safety Commission. 16 CFR Part 1500, 2013. Available at: https://www.cpsc.gov/s3fs-public/pdfs/foia_strongsensitizer.pdf (last accessed 16 August 2017).
- DeLeo V A. P-Phenylenediamine. *Dermatitis* 2006; **17**: 53–55.
- Thyssen J P, White J M L. Epidemiological data on consumer allergy to p-phenylenediamine. *Contact Dermatitis* 2008; **59**: 327–343.
- Hillen U, Jappe U, Frosch P J et al. Late reactions to the patch-test preparations para-phenylenediamine and epoxy resin: a prospective multicentre investigation of the German contact dermatitis research group. *Br J Dermatol* 2006; **154**: 665–670.
- Geier J, Ballmer-Weber B K, Dickel H et al. Monitoring contact sensitization to p-phenylenediamine (PPD) by patch testing with PPD 0.3% in petrolatum. *Contact Dermatitis* 2013; **69**: 26–31.
- Hamann D, Hamann C, Hamann C A. Contemporary Fischer–Maibach investigation: variations in patch test delivery systems and implications for standardization. *Dermatitis* 2013; **24**: 302–312.
- Lachapelle J, Maibach H. *Patch Testing and Prick Testing*, 2nd edition: Berlin, Springer-Verlag, 2009.
- Thyssen J P, Andersen K E, Bruze M et al. P-Phenylenediamine sensitization is more prevalent in central and southern European patch test centres than in Scandinavian: results from a multicentre

- study. *Contact Dermatitis* 2009; **60**: 314–319.
- 9 DeKoven J G, Warshaw E M, Belsito D V et al. North American Contact Dermatitis Group patch test results 2013–2014. *Dermatitis* 2017; **28**: 33–46.
 - 10 Li L F, Liu G, Wang J. Prevalence and 1-year outcome of facial allergic contact dermatitis in patients patch tested in a university hospital. *Contact Dermatitis* 2007; **57**: 187–190.
 - 11 Schnuch A, Lessmann H, Frosch P J, Uter W. para-Phenylenediamine: the profile of an important allergen. Results of the IVDK. *Br J Dermatol* 2008; **159**: 379–386.
 - 12 Hamann D, Yazar K, Hamann C R et al. P-Phenylenediamine and other allergens in hair dye products in the United States: a consumer exposure study. *Contact Dermatitis* 2014; **70**: 213–218.
 - 13 Hextall J M, Alagaratnam N J, Glendinning A K et al. Dose–time relationships for elicitation of contact allergy to para-phenylenediamine. *Contact Dermatitis* 2002; **47**: 96–99.
 - 14 McFadden J P, Wakelin S H, Holloway D B, Basketter D A. The effect of patch duration on the elicitation of para-phenylenediamine contact allergy. *Contact Dermatitis* 1998; **39**: 79–81.
 - 15 Wahlberg J E, Lindberg M. Patch testing. In: *Contact Dermatitis*, 5th edition, Johansen J, Frosch P, Lepoittevin J-P (eds); Berlin, Springer, 2011: pp. 365–390.
 - 16 Johansen J D, Aalto-Korte K, Agner T et al. European Society of Contact Dermatitis guideline for diagnostic patch testing – recommendations on best practice. *Contact Dermatitis* 2015; **73**: 195–221.
 - 17 Friedmann P S. Contact sensitisation and allergic contact dermatitis: immunobiological mechanisms. *Toxicol Lett* 2006; **162**: 49–54.
 - 18 Bruze M, Frick-Engfeldt M, Gruvberger B, Isaksson M. Variation in the amount of petrolatum preparation applied at patch testing. *Contact Dermatitis* 2007; **56**: 38–42.
 - 19 Isaksson M, Gruvberger B, Frick-Engfeldt M, Bruze M. Which test chambers should be used for acetone, ethanol, and water solutions when patch testing? *Contact Dermatitis* 2007; **57**: 134–136.
 - 20 Fischer T, Maibach H. Finn chamber patch test technique. *Contact Dermatitis* 1984; **11**: 137–140.
 - 21 Bruze M, Isaksson M, Gruvberger B, Frick-Engfeldt M. Recommendation of appropriate amounts of petrolatum preparation to be applied at patch testing. *Contact Dermatitis* 2007; **56**: 281–285.
 - 22 Fonacier L. A practical guide to patch testing. *J Allergy Clin Immunol Pract* 2015; **3**: 669–675.
 - 23 Søstød H, Menné T, Johansen J D. Patch test dose–response study of p-phenylenediamine: thresholds and anatomical regional differences. *Contact Dermatitis* 2006; **54**: 145–149.
 - 24 Jensen C D, Paulsen E, Andersen K E. Retrospective evaluation of the consequence of alleged patch test sensitization. *Contact Dermatitis* 2006; **55**: 30–35.
 - 25 Chaudhry H M, Drage L A, El-Azhary R A et al. Delayed patch-test reading after 5 days: an update from the Mayo Clinic contact dermatitis group. *Dermatitis* 2017; **28**: 253–260.
 - 26 Thyssen J P, Menné T, Nielsen N H, Linneberg A. Is there a risk of active sensitization to PPD by patch testing the general population? *Contact Dermatitis* 2007; **57**: 133–134.