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p-Phenylenediamine exposure in real life – a case–control study on sensitization rate, mode and elicitation reactions in the northern Netherlands

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Summary

Background. The prevalence of *p*-phenylenediamine (PPD) sensitization is 4% in the patch tested population and varies between zero and 1.5% in the general population. Hair dye is a recognised sensitization source.

Objectives. To define the prevalence rates of PPD sensitization in the general and patch tested populations of The Netherlands, and to gain insights into relationships between patch test strength and exposure sources.

Methods. Patch test database cases were matched with population-based controls. Analyses were performed based on demographic details, patch test reactions, and potential PPD exposure.

Results. PPD sensitization occurred in 3.3% of the patch tested population and in 1.3% of the general population. Hairdressers had a 4.4-fold increased chance of being sensitized to PPD ($p = 0.041$), and black henna tattoos showed a tendency for a 2.3-fold increased chance of sensitization ($p = 0.081$). Weak elicitation reactions were present in hairdressers and consumers. However, cases who had had black henna tattoos showed significantly more (extremely) strong patch test reactions upon elicitation ($p = 0.015$).

Conclusions. The prevalence rates in the patch tested and general population of The Netherlands are comparable with the prevalence rates of other mid-European centres. PPD sensitization is often attributed to hair dye. However, this study shows that subjects sensitized by black henna tattoos present with very severe elicitation reactions, emphasizing the need for more strict policing the prohibition.

Key words: allergic contact dermatitis; black henna tattoo; contact allergy; hair dye; hairdresser; patch tests; *p*-phenylenediamine; prevalence.

p-Phenylenediamine (PPD, 1,4-diaminobenzene, CAS no. 106-50-3) is an aromatic amine that has been used for over 100 years as a hair dye. It is present in at least 70% of all hair dye products. Because of its low molecular weight of 108 kDa, its strong protein-binding capacity, and its ability to penetrate deeply into the hair shaft, PPD is an extremely effective component, not only in dyeing hair, but also in dyeing textiles, fur, and leather. Moreover, these properties make PPD an extremely potent sensitizer, with the ability to cause severe allergic contact reactions (1). These reactions include acute dermatitis of the scalp and the face, often accompanied by facial oedema, which

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Conflicts of interest: There was no additional external source of funding for this study.

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can be so severe that medical care is necessary. Because of its extreme sensitizing properties, PPD has been considered to be an important safety hazard, and in the past its use was banned in France, Germany and Sweden for some time. The maximum concentration of PPD in the final hair dye product has been 2% in Europe since 2009 (Directive 2009/36/EC).

The prevalence rates of contact allergies to PPD vary widely, and are often derived from the prevalence in a patch tested population. They range from ~1%, calculated within a general population, to >7% in patch tested populations (2, 3). As prevalence rates widely vary and are dependent on the investigated population, the primary aim of this study was to define the prevalence rates of PPD contact allergies in a patch tested population and in the general population in the same geographical area.

The most important risk factor for PPD sensitization seems to be exposure to hair dye. Furthermore, hairdressing is a risk occupation regarding sensitization to PPD. So-called 'temporary black henna' tattoos are frequently described as a serious risk factor for sensitization, and both the clinical presentation and the patch test reaction are often very severe. However, this relationship between black henna and PPD sensitization has only been described in multiple case reports (4, 5). Therefore, the second aim of this study was to gain insights into the exposure to different sources of PPD in sensitized individuals as compared with unsensitized individuals. In addition, this exposure will be related to the severity of the patch test reaction to PPD.

Patients and Methods

Population

In this *post hoc* case–control study, patients with a positive patch test reaction to PPD were selected from the patch test database of the Dermatology Department of the University Medical Center Groningen (UMCG) from January 2007 to March 2014. From this database, information on the grading of the patch test reaction to PPD, demographic information (age and sex), primary sites of complaints and history of atopic dermatitis were extracted.

The control population was the well-defined patch tested population of two municipalities in the northern Netherlands (Groningen, a city, and Stadskanaal, a mostly rural area) who participated in the European Multicentre Fragrance Study of the European Dermato-Epidemiology Network (EDEN). This descriptive epidemiological survey was carried out from August 2008 to October 2011 in six European regions, including the northern Netherlands. The subjects included in this study were randomly recruited by use of a stratified proportional sampling design. All subjects completed a standardized questionnaire on exposure to cosmetics and general skin complaints, such as self-reported atopic complaints (6). A randomly selected subgroup was patch tested with the European baseline series and completed an additional questionnaire on exposure to potential sources of PPD.

Patch testing

Cases and controls were patch tested with the European baseline series, containing PPD (TRUE Test®; Mekos Laboratories AS, Hillerød, Denmark), and readings were performed at D3 according to ICDRG criteria, with an additional reading at D7 for the cases. PPD was tested at a concentration of 0.090 mg/cm^2. Weak (+), strong (++) and extremely strong (+++) positive patch test reactions were considered to be positive.

Hair dye questionnaire

All subjects included in the EDEN Fragrance Study who received a patch test also completed an additional questionnaire. The selected population of PPD-positive patients from the patch test clinic of the UMCG completed the same additional questionnaire. This questionnaire included questions on historical exposure to PPD-containing products, length of exposure, and exposure to PPD in an occupational setting.

Data analyses and statistics

Data were analysed with IBM SPSS® Statistics version 22 for Windows™. A p-value of <0.05 was considered to indicate a statistically significant difference.

The prevalence rates of PPD contact allergies in the general population and the patch tested population were calculated. Demographic and clinical characteristics were compared.

For further analyses, every case was matched with two controls on the basis of both sex and age. Differences in exposure and strengths of the patch tests were described as percentages, and differences were calculated with logistic regression analysis and the chi-square test; odds ratios (ORs) with confidence intervals (CIs) were calculated.

Results

Demographic characteristics

In a 7-year period, 2539 subjects were patch tested with PPD; 84 of these had a positive patch test reaction to PPD. The majority were female (79.5%), and the mean age

was 45.1 years. In total, 49 weak positive (+), 18 strong positive (++) and 17 extremely strong positive (+++) reactions were seen. The prevalence of PPD sensitization was 3.3% (year prevalence range: 2.1–4.7%), with prevalence rates of 3.9% in females and 2.1% in males.

The control population included 478 individuals; 50.6% were female, and the mean age was 45.4 years. Among these 478 individuals, 6 had a positive patch test reaction to PPD, of whom 3 had a weak positive reaction (+) and 3 had a strong positive reaction (++). The prevalence of PPD contact allergies in this sample of the general population was 1.3%; the prevalence in females was 1.7%, and that in males was 0.9%. Within this population, 7.6% had had a patch test before they were included in this study, and almost half of them had had at least one positive reaction to this historical patch test.

Table 1 shows the basic characteristics of all included individuals. Those who did not return the hair dye questionnaire were excluded from further analysis (n = 22). The remaining 62 cases, which were matched with 124 controls, were included in further analyses. There was no statistically significant difference between the age of cases and that of controls ($p = 0.634$). Regarding demographic and clinical characteristics, statistically significant differences were found between atopic individuals, who were overrepresented among the cases (29.7% versus 6.5%, $p < 0.001$), and occupational exposure to PPD (12.9% versus 3.2%, $p = 0.014$). Among the cases, the most frequently involved primary sites were the face, head, and neck, followed by the hands.

Relationship between exposure to PPD-containing products and a contact allergy

Cases were compared with controls to gain insights into differences in their exposure to PPD-containing products, such as hair dye and black henna tattoos (Table 2). Regarding exposure to PPD-containing hair dye products, cases had used hair dye at least once in their lifetime as often as controls (OR 1.33, 95%CI: 0.61–2.90, $p = 0.471$). There was no statistically significant difference in exposure years between cases and controls, as shown in Table 2. The use of hair dye products on facial hair was seen in cases as often as in controls (OR 1.035, 95%CI: 0.52–2.06, $p = 0.922$). Both cases and controls reported reactions to hair dye. However, PPD-positive cases reported such reactions significantly more often than PPD-negative controls (62.9% and 8.1%, respectively; $p < 0.001$; OR 30.81, 95%CI: 11.84–80.21). Moreover, controls reported only mild itching, whereas cases reported more severe reactions, such as oedema of the eyelids and face.

Individuals who had had temporary black henna tattoos showed a tendency to be 2.3-fold more likely to have a PPD contact allergy (95%CI: 0.90–5.89, $p = 0.081$). Although the investigated subgroup was small, hairdressers had a 4.4-fold higher chance of being allergic to PPD (95%CI: 1.06–18.24, $p = 0.041$).

Patch test reaction in relation to exposure

The severity of the patch test reaction to PPD was not associated with exposure to hair dye on the scalp or other body parts, as shown in Table 3. However, subjects with a history of exposure to temporary black henna tattoos had a 14.2-fold higher chance (95%CI: 1.67–121.32, $p = 0.015$) of having strong and extremely strong positive patch test reactions to PPD. When subjects had been exposed to PPD in an occupational setting, they were more likely to have a less severe patch test reaction, although no statistically significant difference between weak and (extremely) strong positive patch test reactions could be shown (OR 0.346, 95%CI: 0.06–1.87, $p = 0.217$).

All individuals who had ever been exposed to temporary black henna tattoos (n = 10) had used hair dye products, including eyelash dye. Nine of them reported an itchy rash after application of the black henna tattoo, and these 9 also had (extremely) strong positive patch test reactions (n = 9 versus n = 1, $p = 0.002$). The 1 subject who had not developed an itchy rash after application of the black henna tattoo had only a weak positive reaction. For the majority (n = 8), the black henna tattoo was probably the first contact with PPD that they ever had, as they had never dyed their hair before (n = 8 versus n = 1, $p = 0.035$).

Discussion

The main aims of this study were to establish the prevalence rates of PPD contact allergy in the general and patch tested populations of the northern Netherlands. Furthermore, we aimed to gain insights into the exposure of both PPD-allergic and non-allergic individuals, and to correlate exposure with the strength of the patch test reaction.

Prevalence rates of PPD sensitization in the patch tested and general populations

The prevalence of PPD contact sensitization in patch tested populations described in the literature varies widely, from ~4% in Germany to up to 7.1% in a UK population (7, 8). The observed prevalence in our patch tested population was 3.3%, which is relatively low as compared with the rates of positive reactions reported by

Table 1. Patch test reactions and demographic and clinical characteristics of cases and controls

	PPD-positive cases, n (%), n = 62	PPD-negative controls, n (%), n = 124
Positive patch test reaction		
+	34 (54.8)	0
++	16 (25.8)	0
+++	12 (19.4)	0
Demographic and clinical characteristics		
Male sex	13 (21.0)	26 (21.0)
Occupation: hairdresser or beautician	8 (12.9)*	4 (3.2)
Atopic eczema	20 (32.3)*	8 (6.5)†
Hand dermatitis	12 (19.4)	NA
Leg dermatitis	1 (1.6)	NA
Face, neck or head dermatitis	35 (56.5)	NA
Age >40 years	41 (66.1)	81 (65.3)
Age in years (mean; median; minimum–maximum)	45.1; 50.0; 13–72	45.4; 50.0; 18–74

NA, not applicable; PPD, *p*-phenylenediamine.
*Statistically significant difference, calculated with the chi-square test.
†Self-reported diagnosis.

Table 2. Relevant exposures to *p*-phenylenediamine-containing products in cases and controls

	Cases, n (%)	Controls, n (%)	*p*-value	OR	95% CI
Hair dye					
Lifetime use	51 (82.3)	94[b] (75.8)	0.471	1.33	0.61–2.90
Duration of use[a]*					
Occasionally/<1 year	11 (17.7)	21 (16.9)	0.937	0.97	0.42–2.21
1–5 years	16 (25.8)	33 (26.6)	0.676	0.86	0.41–1.78
6–10 years	7 (11.3)	14 (11.3)	0.865	0.92	0.35–2.45
>10 years	16 (25.8)	25 (20.2)	0.519	1.28	0.60–2.71
Reaction to hair dye	39[a] (62.9)	10[d] (8.1)	<0.001*	30.81	11.84–80.21
Eyelid or facial oedema	20[a] (32.3)	0[c]	NA	NA	NA
Emergency treatment	15 (24.2)	0	NA	NA	NA
Hospital stay	1 (1.6)	0	NA	NA	NA
Hair dye use on facial hair					
Eyelashes/eyebrows	17[b] (27.4)	34[a] (27.4)	0.922	1.04	0.52–2.06
Beard/moustache	1[b] (1.6)	0	NA	NA	NA
'Temporary black henna' tattoo					
Lifetime use	10[c] (16.1)	10[a] (8.1)	0.081	2.31	0.90–5.89
>1 black henna tattoo	4	0	NA	NA	NA
Reaction to tattoo	9	0	NA	NA	NA
Occupation					
Hairdresser	6[a] (9.7)	3 (2.4)	0.041*	4.40	1.06–18.24
Beautician	2[b] (3.2)	2 (1.6)	0.463	2.10	0.29–15.31

CI, confidence interval; NA, not applicable; OR, odds ratio.
Missing values: [a]1, [b]2, [c]3, [d]5.
*Statistically significant difference, calculated with logistic regression analysis.

other European centres (9). Our lower prevalence could be related to the high number of blond-haired individuals in the northern Netherlands. PPD and its related substances are more often present in higher percentages in the darker shades of hair dye products. Thyssen et al. previously described this phenomenon (10). He found a lower sensitization rate in Scandinavian cities than in central and southern European cities, in which a higher concentration of dark-haired people is present. It is also known that Asian people, who often dye both scalp and facial hair, are more frequently sensitized to PPD, which substantiates this assumption (11).

The prevalence of PPD contact allergies in the general population is rarely described, as most studies are performed in patch tested populations. Prevalence rates in the general population are frequently derived from the patch tested population, instead of observed prevalence rates, as in the present study. These derived prevalence

Table 3. Exposure to *p*-phenylenediamine (PPD)-containing products in the different subgroups classified by the strength of the patch test reaction to PPD

Exposure source	+ (n = 34)	++/+++ (n = 28)	*p*-value	OR	CI
Hair dye	26 (76.5)	25 (89.3)	0.199	2.56	0.61–10.78
Hair dye use on facial hair[a]	10 (31.3)	7 (25)	0.593	0.73	0.24–2.28
'Temporary black henna' tattoo	1 (3.2)	9 (32.1)	0.015*	14.21	1.67–121.32
Occupation (hairdresser)	4 (17.6)	2 (7.1)	0.217	0.35	0.06–1.87

CI, confidence interval; OR, odds ratio.
Missing values: [a] 2.
*Statistically significant difference, calculated with the chi-square test.

rates in northern European countries are all ~1% (12, 13). Observed prevalence rates vary widely, ranging from zero to 1.5% overall in Europe. The prevalence of 1.3% in our general population is in line with the observed prevalence of 1.5% found by Schäfer et al. (14). The prevalence of contact allergy to PPD in the general population of Asia is twice as high as the prevalence rates found in the present study and in Europe, that is, 2.7% as found in the study among Thai adults by White et al. in 2007 (15). This difference can be attributed to the difference in hair dye behaviour as described previously.

Atopic individuals among the patch tested population

Among the controls included in this study, a relatively small number reported the presence of atopic dermatitis during their lifetime (6.5%). The lifetime prevalence of atopic dermatitis as described in the literature is ~20% (16). This could be attributable to the recall bias that is introduced when adults are asked about events in early childhood. However, only 3 individuals (2.4%) reported recent active manifestations of atopic dermatitis. This percentage is in line with the adult prevalence of atopic dermatitis.

The percentage of individuals with actual atopic dermatitis among the cases was statistically significantly higher than among the controls. The difference could be attributable to selection bias. An explanation for this could be that patch testing is often performed as a part of the routine diagnostic procedure for patients with recalcitrant atopic dermatitis.

Another, but controversial, hypothesis is that the diminished skin barrier in atopic individuals allows allergens to penetrate the stratum corneum more easily. In the case of contact allergies to PPD, reactive oxygen species (ROS) are produced when it is applied to the hair and skin. These ROS are able to compromise the epidermal barrier, leading to increased penetration of a hapten. Niwa et al. described increased ROS production in patients with atopic dermatitis, which could contribute to more compromised barrier function and, hypothetically, to easier penetration of hapten, in addition to the ROS produced during contact allergic reactions to PPD (17).

The length of exposure to hair dye does not increase the sensitization risk

Assuming that exposure to hair dye is more frequent in PPD-sensitized individuals, we looked at exposure to hair dye products during the individual's lifetime as well as the length of exposure in years. No difference could be shown. However, as a question regarding the frequency of exposure to hair dye in either an occupational setting, at the hairdressers or at home was not included in the questionnaire, no statements can be made regarding this point.

It is common knowledge that hairdressers are at risk of being sensitized to PPD. In the present study, the chance of being sensitized as a hairdresser was statistically significantly increased, by 4.4-fold. This might be explained by their much higher frequency of exposure. Moreover, hairdressers have higher exposure to irritant factors, such as water, detergents, and chemicals, which impair skin barrier function (18).

In this case–control study, beauticians were not at risk of sensitization. This finding could be related to the small numbers of beauticians in our population, which hampers the drawing of meaningful conclusions. Another cause could be that beauticians in The Netherlands are probably less exposed to PPD in products intended for dyeing facial hair.

The use of hair dye on facial hair (eyelashes, eyebrows, moustaches, and beards) is often regarded as a risky event, and in the United States eyelash colorants were banned after the 'lash lure' scandal in 1937 (19). However, in our study, we found that, in consumers, exposure to eyelash dye and other dyes intended for dyeing facial hair did not increase the risk of sensitization.

A significant source of PPD is temporary black henna tattoo. The small group of individuals who had had a black henna tattoo showed a tendency to have a 2.3-fold increased chance of being sensitized to PPD

($p = 0.081$). This suggests that black henna tattoos also constitute a risk factor for sensitization. A wide variety of, mostly high, PPD concentrations is seen, because black henna is not a standardized material (20). High PPD concentrations vastly increase the sensitizing potential of black henna tattoo. Kligman et al., who showed that all 24 experimental volunteers exposed to 10% PPD were sensitized, previously described this phenomenon (21).

The severity of the patch test reaction is related to the mode of sensitization

Whenever the clinical manifestations of contact allergy to PPD are considered, one could suggest that different allergic subtypes can be distinguished. Some individuals with a PPD contact allergy present with a history of a mild itchy rash and probably some erythema on the scalp and scalp margin, often resolving within a few days. These individuals often have weak positive patch test reactions. Over 70% of them will continue to dye their hair after they have been diagnosed with contact allergy to PPD, as described by Ho et al. (22).

Another subtype of PPD-sensitized individuals comprises those who present with very severe complaints of oedema of the eyelids and face, sometimes accompanied by swelling of the mucosa of the nose and throat. Patch test reactions are often strong to extremely strong positive. After one episode of such a severe allergic reaction, the majority of those patients never use hair dye products again.

Moreover, similar differences between mild and strong to extremely strong positive patch test reactions were not found for other PPD sources, such as occupational exposure in hairdressers, who actually have a lower chance of having strong to extremely strong positive reactions during elicitation. From previous research, it is clear that frequent exposure to low concentrations of an allergen, for example 2,4-dinitrochlorobenzene, can cause sensitization that is comparable to sensitization caused by one dose of the allergen at a very high dose (23). However, it is also known that a high sensitization dose is more likely to cause a high degree of sensitization, resulting in a lower concentration of the sensitizer being required to elicit a reaction (24). Due to the use of gloves during the hair dye procedure, hairdressers are exposed to very low concentrations of PPD. Probably, a weak degree of sensitization occurs, resulting in less severe elicitation reactions on re-exposure. This makes repeated exposure to low doses a risk factor for the development of weak elicitation reactions.

In our study, we found a relationship with historical exposure to temporary black henna tattoos; individuals exposed to a black henna tattoo had a 14-fold higher chance of having a strong or extremely strong positive patch test reaction. Black henna tattoos are known for their sensitizing potential, but, up to now, it has been not completely clear why this elicits more severe contact allergic reactions. Hostynek et al. have shown that, after sensitization with a high dose of allergen, the reaction upon elicitation tends to be more severe (25). Although these studies were not performed with PPD, the same mechanism is applicable to this allergen, as described by Spornraft-Ragaller et al., who clarified the relationship between temporary black henna tattoos and patch test reactivity to PPD in children. They concluded that alarmingly high percentages of extremely strong patch test reactions were observed. Sensitization in those cases was probably caused by PPD in black henna tattoos (26). Our results are in line with previous findings that high doses of allergen not only induce sensitization, but also make the individual react to a lower dose of allergen upon challenge.

Limitations of this study

As the exposure assessment in this study is based only on questionnaires, there is a risk of recall bias, especially when the selected and questioned population stopped using hair dye products after a diagnosis of PPD contact allergy many years ago. Case–control studies are known for their risk of selection bias. The selection of cases might be biased, because individuals with atopic dermatitis could be more often patch tested, owing to their clinical presentation, for example head and neck dermatitis. This could explain the higher percentage of atopic individuals among cases. These results are in line with the percentage of atopic dermatitis among patch tested subjects having a contact allergy within our clinic in Groningen (unpublished results). The control population could be biased because they were recruited with invitation letters. Selection bias could also be introduced because individuals with skin complaints are often more prone to participate in research regarding their disease. With the use of these letters, there is a risk of unwanted exclusion of certain groups, such as people who have little knowledge of the language, youngsters, or elderly people. Furthermore, possible confounders, such as socio-economic status and level of education, were not accounted for.

Whenever contact allergy to PPD without a history of exposure is investigated, one should also search for exposure to cross-reacting allergens, which can cause a positive PPD patch test. Although cross-reactions are of great interest, they are outside the scope of this investigation.

Concluding remarks

In the present study, evidence has been given not only of PPD contact allergies resulting from consumer exposure to hair dye products, but also of allergies resulting from the professional use of PPD-containing products. In spite of European regulation, PPD remains an important allergen, because of its extreme sensitizing properties. Sensitization to PPD can occur because of prolonged exposure at a high frequency, as with hairdressers, and causes predominantly weak positive elicitation reactions. Moreover, sensitization with a single high dose of PPD, as in temporary black henna tattoos, causes mostly strong to extremely strong positive allergic reactions. This emphasizes the need for more strict regulations and enforcement of the prohibition of PPD in the dye of black henna tattoos.

References

1 Basketter D A, Goodwin B F. Investigation of the prohapten concept. Cross reactions between 1,4-substituted benzene derivatives in the guinea pig. *Contact Dermatitis* 1988: **19**: 248–253.

2 Krasteva M, Bons B, Ryan C, Gerberick G F. Consumer allergy to oxidative hair coloring products: epidemiologic data in the literature. *Dermatitis* 2009: **20**: 123–141.

3 Thyssen J P, White J M, European Society of Contact Dermatitis. Epidemiological data on consumer allergy to p-phenylenediamine. *Contact Dermatitis* 2008: **59**: 327–343.

4 de Groot A C. Side-effects of henna and semi-permanent 'black henna' tattoos: a full review. *Contact Dermatitis* 2013: **69**: 1–25.

5 Kind F, Scherer K, Bircher A J. Contact dermatitis to para-phenylenediamine in hair dye following sensitization to black henna tattoos – an ongoing problem. *J Dtsch Dermatol Ges* 2012: **10**: 572–578.

6 Rossi M, Coenraads P J, Diepgen T et al. Design and feasibility of an international study assessing the prevalence of contact allergy to fragrances in the general population: the European Dermato-Epidemiology Network Fragrance Study. *Dermatology* 2010: **221**: 267–275.

7 Patel S, Basketter D A, Jefferies D et al. Patch test frequency to p-phenylenediamine: follow up over the last 6 years. *Contact Dermatitis* 2007: **56**: 35–37.

8 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.

9 Uter W, Aberer W, Armario-Hita J C et al. Current patch test results with the European baseline series and extensions to it from the 'European Surveillance System on Contact Allergy' network, 2007–2008. *Contact Dermatitis* 2012: **67**: 9–19.

10 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.

11 Chan Y C, Ng S K, Goh C L. Positive patch-test reactions to para-phenylenediamine, their clinical relevance and the concept of clinical tolerance. *Contact Dermatitis* 2001: **45**: 217–220.

12 Nielsen N H, Menné T. Allergic contact sensitization in an unselected Danish population. The Glostrup Allergy Study, Denmark. *Acta Derm Venereol* 1992: **72**: 456–460.

13 Thyssen J P, Linneberg A, Menné T, Nielsen N H, Johansen J D. Contact allergy to allergens of the TRUE-test (panels 1 and 2) has decreased modestly in the general population. *Br J Dermatol* 2009: **161**: 1124–1129.

14 Schäfer T, Bohler E, Ruhdorfer S et al. Epidemiology of contact allergy in adults. *Allergy* 2001: **56**: 1192–1196.

15 White J M, Gilmour N J, Jeffries D et al. A general population from Thailand: incidence of common allergens with emphasis on para-phenylenediamine. *Clin Exp Allergy* 2007: **37**: 1848–1853.

16 Thomas B R, White I R, McFadden J P, Banerjee P. Positive relationship – intensity of response to p-phenylenediamine on patch testing and cross-reactions with related allergens. *Contact Dermatitis* 2014: **71**: 98–101.

17 Niwa Y, Sumi H, Kawahira K, Terashima T, Nakamura T, Akamatsu H. Protein oxidative damage in the stratum corneum: evidence for a link between environmental oxidants and the changing prevalence and nature of atopic dermatitis in Japan. *Br J Dermatol* 2003: **149**: 248–254.

18 Brans R, Merk H F. Aspects of occupational skin diseases in hair dressers. *Hautarzt* 2009: **60**: 973–978.

19 US Food and Drug Administration Home Page. A Century of Ensuring Safe Foods and Cosmtics. Available at: www.fda.gov. (last accessed 01 September 2014).

20 Brancaccio R R, Brown L H, Chang Y T, Fogelman J P, Mafong E A, Cohen D E. Identification and quantification of para-phenylenediamine in a temporary black henna tattoo. *Am J Contact Dermat* 2002: **13**: 15–18.

21 Kligman A M. The identification of contact allergens by human assay. 3. The maximization test: a procedure for screening and rating contact sensitizers. *J Invest Dermatol* 1966: **47**: 393–409.

22 Ho S G, Basketter D A, Jefferies D, Rycroft R J, White I R, McFadden J P. Analysis of para-phenylenediamine allergic patients in relation to strength of patch test reaction. *Br J Dermatol* 2005: **153**: 364–367.

23 Paramasivan P, Lai C, Pickard C, Ardern-Jones M, Healy E, Friedmann P S. Repeated low-dose skin exposure is an effective sensitizing stimulus, a factor to be taken into account in predicting sensitization risk. *Br J Dermatol* 2010: **162**: 594–597.

24 Friedmann P S. The relationships between exposure dose and response in induction and elicitation of contact hypersensitivity in humans. *Br J Dermatol* 2007: **157**: 1093–1102.

25 Hostynek J J, Maibach H I. Thresholds of elicitation depend on induction conditions. Could low level exposure induce sub-clinical allergic states that are only elicited under the severe conditions of clinical diagnosis? *Food Chem Toxicol* 2004: **42**: 1859–1865.

26 Spornraft-Ragaller P, Schnuch A, Uter W. Extreme patch test reactivity to p-phenylenediamine but not to other allergens in children. *Contact Dermatitis* 2011: **65**: 220–226.