

Are gloves sufficiently protective when hairdressers are exposed to permanent hair dyes? An *in vivo* study

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doi:10.1111/cod.12320

Summary

Background. The use of permanent hair dyes exposes hairdressers to contact allergens such as *p*-phenylenediamine (PPD), and the preventive measures are insufficient.

Objectives. To perform an *in vivo* test to study the protective effect of gloves commonly used by hairdressers.

Patients/materials/methods. Six gloves from Sweden, Italy and Germany were studied: two vinyl, one natural rubber latex, two nitrile, and one polyethylene. The hair dye used for the provocation was a dark shade permanent dye containing PPD. The dye was mixed with hydrogen peroxide, and 8 PPD-sensitized volunteers were tested with the gloves as a membrane between the hair dye and the skin in a cylindrical open chamber system. Three exposure times (15, 30 and 60 min) were used.

Results. Eczematous reactions were found when natural rubber latex, polyethylene and vinyl gloves were tested with the dye. The nitrile gloves gave good protection, even after 60 min of exposure to the hair dye.

Conclusions. Many protective gloves used by hairdressers are unsuitable for protection against the risk of elicitation of allergic contact dermatitis caused by PPD.

Key words: hairdressers; permanent hair dyes; *p*-phenylenediamine; protective gloves.

Hairdressers are at risk of acquiring occupational dermatitis (1). The group is exposed to irritant factors, including both physical and chemical factors, as well as a wide range of chemicals used in hair treatments that can cause contact allergy and resulting dermatitis. *p*-Phenylenediamine (PPD) is an extreme skin sensitizer, and has been reported to be one of the main permanent hair dye allergens, both for consumers and in the occupational setting (2–4). Unfortunately, the need to

use protective gloves may not be emphasized enough, the use may be inadequate, and the intrinsic protective capacity of gloves varies. Studies have been performed with standardized *in vitro* methods (5–8) to evaluate the permeation of oxidative hair dye compounds through gloves, with contrasting results.

The aim of the present study was to evaluate *in vivo* the efficacy of protective gloves used by hairdressers in three different European countries (Sweden, Italy, and Germany), by simulating their ordinary exposure to oxidative hair dyes during hair colouring. Accordingly, we used an *in vivo* method developed by Andersson and Bruze (9) to evaluate the protective properties of gloves against an oxidative hair dye containing PPD, in subjects already sensitized to PPD, simulating their regular use as protection during the performance of an occupational task.

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Funding: None.

Conflicts of interest: The authors declare no conflicts of interest.

Accepted for publication 14 October 2014

Table 1. Gloves tested in the study; data are according to the manufacturers/suppliers, with the exception of the thickness on palm/back of gloves, which was measured at our laboratory

Glove	Gloves (commercial name)	Manufacturer/supplier	Thickness (mm) *	Material
A	Medical Exam gloves®	Abena A/S, Aabenraa, Denmark	0.034	Vinyl
B	Guanto Monouso®	M.A.RE.B., Milano, Italy	0.029	Polyethylene
C	Guanti in lattice®	Ro.ial., Prato, Italy	0.038	Natural rubber latex
D	Walking innovative®	Brenta, Venezia, Italy	0.035	Nitrile
E	Alfatex 30 Nitril blau®	Sänger GmbH, Schrozberg, Germany	0.040	Nitrile
F	Vinyl 300®	Latexha W. Nentwich, Diepolsau, Switzerland	0.037	Vinyl
Negative control	Silver Shield 4H®	North Safety Products by Honeywell, Smithfield, RI, USA	0.04	Polyethylene-ethylene-vinyl-alcohol

*Mean value of three measurements.

Materials and Methods

Workplace visit

The normal work of hairdressers when colouring hair was observed by visiting a hairdresser's salon and a school for hairdressers' apprentices located in Malmö, Sweden. Special attention was given to protective measures used by the hairdressers and to the amount of time spent on different tasks. The information gathered in this small group was compared with that available in the literature concerning hairdressers working in other countries, with the aim of achieving similar exposure conditions to permanent hair dyes for the study as those that the hairdresser has during a normal workday (10–12).

Subjects

Eight subjects (7 females and 1 male, aged 20–61 years, median 37 years) were enrolled as volunteers in the study. They had been previously patch tested at the Department of Occupational and Environmental Dermatology, Skåne University Hospital, and had shown ++ or +++ reactions to PPD 1% pet., read according to ICDRG criteria. They had been sensitized either through occupational use or as consumers using hair dyes or so-called black 'temporary henna' tattoos. All of them took measures against exposure to PPD in their personal life, and there had been no recent reactions to products used for skin or hair care.

Gloves

The gloves used were of different materials: vinyl, natural rubber latex, nitrile, and polyethylene. They were

obtained from hairdressing product retailers or from hairdressers in three European countries (Sweden, Italy, and Germany). The gloves were selected either because they were recommended as protective gloves by the local hairdressers' union, or had been recommended by shops where hairdressers bought gloves and work equipment. For each different type of glove, we measured the thickness at the palm/back of three samples by using a micrometre screw-gauge. The average thickness was within a range of 0.029–0.040 mm (Table 1). Among 24 samples collected we selected 6 (2 vinyl, 1 natural rubber latex, 2 nitrile and 1 polyethylene). The 4H® glove (made of layers of polyethylene and ethylene-vinyl-alcohol) (North Safety Products by Honeywell, Smithfield, RI, USA) was used as a negative control because of its known ability to protect against many chemicals (9).

Hair dyes

A permanent hair dye with a black shade sold in Sweden for use by hairdressers was used. The ingredient labelling of the product complied with the requirements of the European Cosmetics Directive (13). High-performance liquid chromatography analysis of the hair dye samples was performed at the laboratory of the Department of Occupational and Environmental Dermatology in Malmö to establish the PPD content (1.8%) in the colouring cream of the hair dye (14).

The ingredients labelled on the package are listed in Table 2. The hair dye was prepared, according to the product instructions, by mixing the colouring cream containing PPD with the developer cream containing hydrogen peroxide 3% at equal parts (1:1). The initial PPD concentration in the final testing product was thus 0.9%.

Table 2. Ingredients listed on the label of the hair dye Black Infiniti Affinage®, shade 1.0 (International Hair Cosmetics Ltd, Romsey, UK)

Chemicals labelled on the colouring cream

4-Amino-2-hydroxytoluene, 2-amino-3-hydroxypyridine, *N,N*-bis(2-hydroxyethyl)-*p*-phenylenediamine sulfate, ammonia, resorcinol, *p*-methylaminophenol sulfate, *p*-phenylenediamine, *p*-aminophenol, 2-amino-4-hydroxyethylaminoanisole sulfate, 4-chlororesorcinol, 2-methylresorcinol, 4-amino-*n*-cresol, 6-amino-*n*-cresol, HC Red No. 3, HC Yellow No. 2, HC Yellow No. 4, HC Blue No. 2, *p*-aminophenol, laureth-3, disodium EDTA, parfum, sodium hydrosulfite, sodium sulfite, *DATEM™, cetareth-25, cocamidopropyl betaine, propylene glycol, cocamide MEA, glycol stearate, myristyl alcohol, aqua

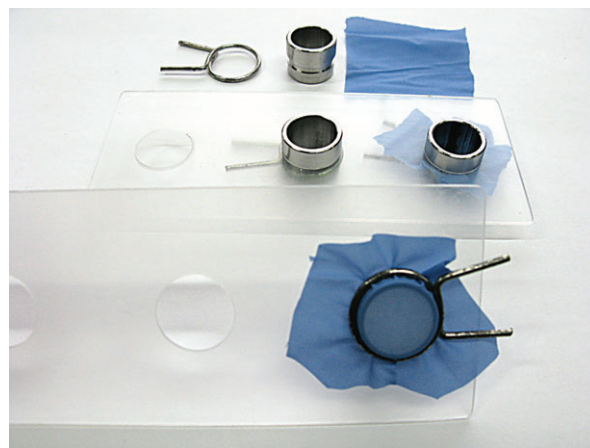
*Diacyl tartaric acid ester of monoglycerides and diglycerides, E472, emulsifier.

Patch test

To establish the current reactivity of each volunteer, a dilution series of PPD in acetone (1.0%, 0.1%, 0.01%, 0.001%, and 0.0001%) was patch tested at the same time as the *in vivo* study was performed. Finn Chambers® (Ø 8 mm) (Smart Practice, Phoenix, AZ, USA) on Scanpor® tape (Norgesplaster A/S, Vennessla, Norway) were used for patch testing with the dilution series. Fifteen microlitres of the test solution was applied with a micropipette to the filter paper disc in each test chamber. Acetone was used because exact dilutions series cannot be achieved with pet.. The chambers were placed on the back of the patients at the same time as the *in vivo* study was performed, and left in place for 48 hr; readings were performed on D4 and D7. The reactions were scored according to ICDRG guidelines.

In vivo 'penetration test'

An open chamber system developed at the Department of Malmö (9), and already used to test the efficacies of different glove materials in protecting against chemicals (15), was used. A group of three circular chambers (12 mm in height; 12 mm in inner diameter) made of chemical-resistant stainless steel was fitted in a 1-mm-thick flexible acrylic support. Rectangular pieces of each glove, cut from the palm/back, were fixed with a nickel-free metallic clip to the chambers, thus constituting the bottom of the chamber (Fig. 1). The glove material acted as a membrane between the chamber and the skin. Three different glove materials were simultaneously tested for each of the three chamber's support devices. The subjects lay horizontally on a massage bed, and the testing system was taped to the skin of the back with Scanpor® tape (Fig. 2). The chambers were filled up to half-height (~ 6 mm) with the hair dye/peroxide mixture.

**Fig. 1.** The 'open chamber' test system used.

The mixture was prepared within 4 min of application. Three exposure times (15, 30 and 60 min) were chosen to mimic the range of time that ordinary hair dyeing procedures can take. For hair dyeing, hairdressers generally wear the same pair of gloves, and the time rarely exceeds 60 min. Six gloves commonly used by hairdressers were tested together on the back of each subject at three exposure times. As a negative control, the 4H® glove was used with an exposure time of 60 min; as a positive control, a chamber devoid of glove bottom filled with the hair dye directly in contact with the skin was used with the same exposure time. In total, each individual was tested with 20 chambers. After removal of the testing devices at three exposure times, a clinical examination of the skin corresponding to the glove test application area was performed to detect the presence of dark spots as visible signs of penetration of the hair dye through the gloves. The integrity of the pieces of glove tested and the possible presence of breakage were checked by cleansing the glove material and visual examination. Readings of the areas were performed according to ICDRG guidelines on D2, D4, and D7. The test reactions were also photo-documented.

Ethics

The subjects gave informed consent, and the Regional Ethics Committee for Human Research of Skåne University Hospital, Sweden, approved the protocol (No. 487/2011). The study was conducted from October to November 2011.

Statistics

McNemar's test (two-sided) was used to compare the protective effects of the different gloves pairwise. A one-sided

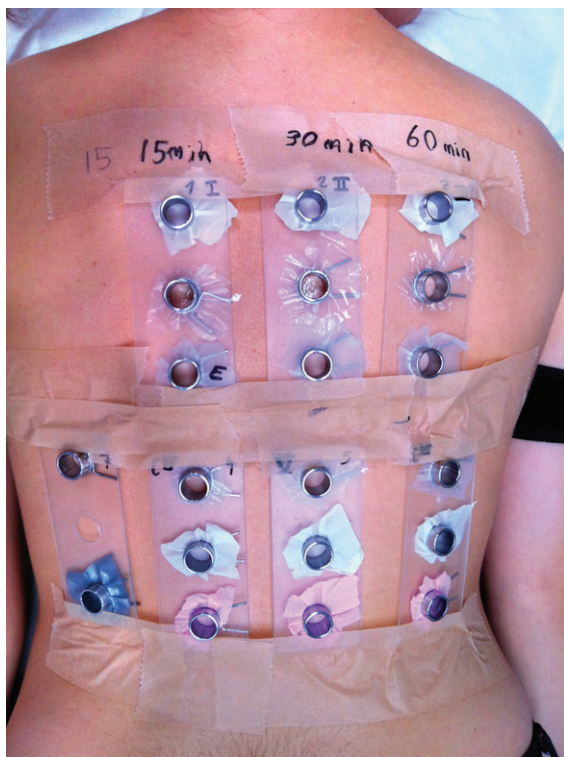


Fig. 2. Testing device before the application of the hair dye mixed with hydrogen peroxide. Each glove material was tested at three exposure times (15, 30 and 60 min). The positive and the negative control (on the left side) were tested for 60 min. Row 1: glove A (vinyl). Row 2: glove B (polyethylene). Row 3: glove C (natural rubber latex). Row 4: glove D (nitrile). Row 5: glove E (nitrile). Row 6: glove F (vinyl). Left side, upper: positive control (no glove). Left side, lower: negative control (4H[®] glove).

McNemar's test was used to compare the protective effect of each individual glove with that of the 4H[®] glove (negative control). A p -value of <0.05 was considered to be significant. The relationship between the degree of reactivity to PPD and the number of hair dye/glove reactions was compared by use of Fisher's exact test (two-sided), dichotomizing the 8 subjects into those with PPD thresholds of 0.1% and 1.0% in one group, and those with a threshold of 0.01% in the other group. Spearman's rank correlation test was used to investigate the association between degree of reactivity and the number of positive reactions at the three exposure times.

Results

Serial dilution testing with PPD in acetone showed positive reactions to concentrations as low as 1.0% in 2 subjects: at 0.1% in 1 and at 0.01% in 5 (threshold of reactivity) (Table 3). The *in vivo* test was well tolerated, and

no symptoms appeared during the procedure. No black spots were observed on the backs of the 8 subjects after removal of the glove tests. The results of the glove testing are summarized in Table 3. Considerable differences in the protective capacities of different glove materials were observed. For the 4H[®] glove (Glove H), with an exposure time of 60 min, no skin reactions were detected in any subject. For the two different nitrile gloves, none of the 8 PPD-sensitized subjects reacted at any exposure time. The nitrile gloves thus showed the same protective effect as the negative control. One of the two vinyl gloves (glove F; Table 1) showed the least protection in terms of number of reactions developed at the various exposure times: one at 15 min, three at 30 min, and six at 60 min. There were fewer positive reactions to the other three gloves – glove A (vinyl), glove B (polyethylene), and glove C (natural rubber latex) – than to glove F (vinyl). There were statistically more skin reactions to all gloves, except for nitrile gloves (D and E), at 60 min ($p > 0.3$ for gloves D and E; $p = 0.031$ for gloves A, B, and C; $p = 0.016$ for glove F) than to the negative control glove. The pairwise comparisons between the six gloves showed significant differences between the respective nitrile glove on one hand and glove F on the other ($p = 0.031$). The corresponding comparisons between the respective nitrile glove and gloves A, B and C were non-significant ($p = 0.063$). Stronger and more numerous reactions to the penetration tests were seen in the 5 subjects reacting to lower concentrations of PPD in acetone at 0.01% than in the subject reacting to 0.1% and the 2 subjects reacting to 1.0%. The dichotomized comparisons at the exposure times of 60 and 30 min resulted in significant differences ($p < 0.001$), whereas the comparison at 15 min was non-significant ($p = 0.071$). One of the patients with a positive reaction to 1.0% PPD did not react to the penetration tests at all. Seven of the 8 subjects enrolled showed positive skin reactions to the glove penetration tests and to the positive control consisting of the hair dye placed directly on the skin. All of the patients reacting in the study to the glove penetration test showed a dose–response relationship between increasing exposure times and the number of positive reactions to the glove penetration test (Figs. 3 and 4), with a significant correlation at the 0.01 level for the exposure time of 60 min (Spearman's rank correlation test).

Discussion

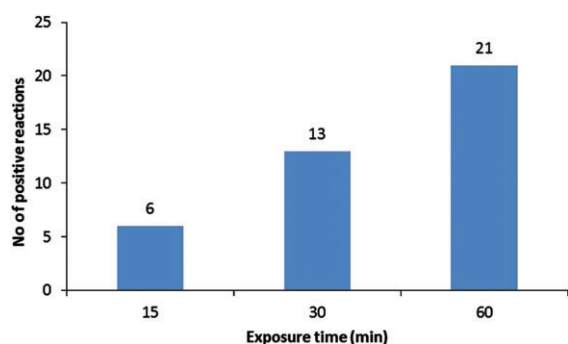
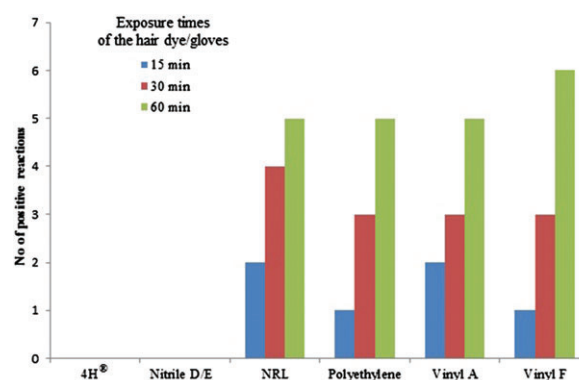
In this study, the protective effect of gloves used by hairdressers in Europe (Italy, Sweden, and Germany) with regard to elicitation of allergic contact dermatitis caused by PPD was studied with an *in vivo* system simulating work exposure. The *in vivo* study showed significant differences between the six glove materials tested and good

Table 3. Results of test reading on D4/D7 of the open exposure test with hair dye/gloves among 8 *p*-phenylenediamine (PPD)-sensitized subjects and their thresholds of reactivity

Subject number		1*	2	3	4*	5	6	7	8
Threshold of reactivity to PPD dilution series (%)		0.01	1.0	1.0	0.1	0.01	0.01	0.01	0.01
Exposure times (min)									
Gloves									
A (vinyl)	15	+++	—	—	—	—	—	(+)	+
	30	+++	—	—	—	—	—	++	+
	60	+++	—	—	(+)	+++	+++	+++	+
B (polyethylene)	15	++	—	—	—	—	—	—	—
	30	++	—	—	—	—	—	+	+
	60	++	—	—	—	++	++	++	+
C (natural rubber latex)	15	+++	—	—	—	—	—	—	++
	30	+++	—	—	—	++	—	++	+
	60	+++	—	—	—	+++	+++	+++	+
D (nitrile)	15	—	—	—	—	—	—	—	—
	30	—	—	—	—	—	—	—	—
	60	—	—	—	—	—	—	—	—
E (nitrile)	15	—	—	—	—	—	—	—	—
	30	—	—	—	—	—	—	—	—
	60	—	—	—	—	—	—	—	—
F (vinyl)	15	—	—	—	—	—	—	—	+
	30	++	—	—	—	++	—	(+)	+
	60	++	—	—	+++	+++	++	+++	++
G (no-glove positive control)	60	+++	+++	—	+++	+++	+++	+++	++
H (4H [®] glove negative control)	60	—	—	—	—	—	—	—	—

The lowest value of the PPD dilution series responsible for a positive reaction is included in the table. The highest score of positive reactions at D2/D4/D7 has been selected.

* = D2 test reading.

**Fig. 3.** Dose–response trend of the positive reactions to the hair dye permeating through the gloves with increasing numbers of positive reactions at longer exposure times.**Fig. 4.** Number of positive reactions to the hair dye permeating through the different gloves at three exposure times.

protective ability of the nitrile gloves that was comparable to that of the 4H[®] glove used as a negative control. The vinyl, polyethylene and natural rubber latex gloves were poorly protective, as shown by the occurrence of positive test reactions, the intensity of which was related to increasing exposure time and to the threshold of the PPD reactivity of the subjects (Figs. 4 and 5).

For many at-risk occupations, gloves are the best means for protection, even though glove use is a risk factor in itself (16–19). In the occupational field, the type of glove used must depend on the nature of the chemicals involved (20), but the chemical resistance of each glove material is also influenced by the exposure time

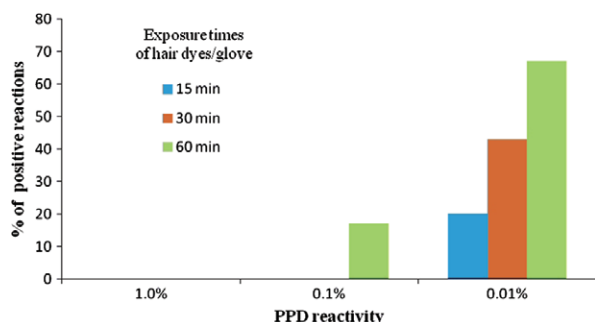


Fig. 5. Association between the individual degree of patch test reactivity to *p*-phenylenediamine (PPD) and percentage of positive reactions to hair dye for the six gloves with three different exposure times.

and the conditions under which the work is performed. Standard *in vitro* test methods have been developed in the United States and Europe to assess the protective efficacy of gloves against different chemicals (7, 8), and are performed by the manufacturers before marketing. Previous *in vitro* studies assessed the resistance to permeation by permanent hair dye chemicals through protective gloves (21). Lind et al. (5) observed considerable protection by the gloves tested (natural rubber latex, polyvinylchloride, nitrile, and polyethylene) against PPD, toluene-2,5-diamine sulfate and resorcinol without adding hydrogen peroxide, that is, without the actual exposure seen when using the glove. The gloves were tested for up to 4 hr, and all of the materials withstood permeation well, giving protection for ≥ 30 min. Lee and Lin (21) investigated *in vitro* the permeation behaviour of *p*-aminophenol, *m*-aminophenol, *o*-aminophenol and PPD in single and mixed challenge solutions (with ethanol or hydrogen peroxide) with disposable natural rubber latex, polyvinylchloride and neoprene gloves. Their results showed that hydrogen peroxide did not accelerate hair dye permeation through the gloves, that natural rubber latex and polyvinylchloride were not recommended for repeated handling of permanent hair dyes, and that neoprene gloves gave good protection for at least 8 hr.

The *in vitro* methods are not expected to represent all conditions found in normal working circumstances. The protective efficacy of gloves can be influenced by factors such as occlusion, sweating, stretching, and skin temperature (15). We chose an *in vivo* study that was aimed at reproducing the actual daily exposure of hairdressers to permanent hair dyes as used at work, that is, mixed with hydrogen peroxide.

Hair dyeing can take a variable amount of time, depending on hair length and the type of processing, and,

including the time spent preparing the dye and applying it, can take > 1 hr. We aimed at reproducing this by using different exposure times in an *in vivo* system. We observed at our visit to a hairdresser's salon, as has been found in previous studies, that most hairdressers wear gloves when applying permanent hair dyes. However, it has been seen in several studies that they often wear the same pair of gloves for more than one task, or reuse disposable gloves after washing them in a washing machine with towels and drying them in a tumble dryer (10, 22, 23). Even turning the glove inside out before use has been observed. This work practice may give rise to longer exposure times and, possibly, contaminated gloves.

It is important for the glove to be chosen both to minimize the risk factors associated with wearing them and to give sufficient protection for the task to be performed. Making a bad choice of gloves might actually expose users to more contact with the substance against which the glove was intended to be protective, as users might consider themselves to be protected and therefore have prolonged exposure times. If a product is uncoloured, it might be difficult to suspect that permeation occurs.

In our evaluation, the vinyl, polyethylene and natural rubber latex gloves were insufficiently protective, being permeated by contact allergens from the hair dye after an exposure time of 15 min, and the number of positive test reactions increasing with increasing exposure time (Fig. 6). In this study, both exposure time and the reactivity of the individual were taken into account, as the study was performed as a provocation study.

The effect of the PPD reactivity can be clearly seen in our results, as the subjects with the lowest reactivity



Fig. 6. Positive reactions in subject no. 1 at the D2 test reading.

had fewer and weaker reactions to the allergens that had permeated through the gloves than the subjects with high reactivity. Subject no. 3 with a threshold of reactivity to PPD of 1.0% did not react to any glove tests or to the positive control. Possible explanations for the lack of reactions are too weak reactivity to PPD with only 0.9% present in the mixture when the oxidation starts, and/or less cross-reactivity to the oxidation products of PPD. In the oxidation process that occurs when a hair dye is used, new chemicals are formed, and the patterns of reactivity to these oxidation products in patients diagnosed with allergy to PPD (not oxidized) are known to differ (24), which might influence results.

With an exposure time of 1 hr, this study shows that the two nitrile gloves tested are as protective against the PPD-containing hair dye mixed with hydrogen peroxide as the 4H[®] glove. On the other hand, the investigated vinyl, polyethylene and natural rubber latex gloves were insufficiently protective. For these gloves, longer exposure times and higher PPD reactivity resulted in more positive hair

dye glove test reaction, that is, a higher risk for allergic contact dermatitis. One could, of course, argue that our results indicate that, for patients with low PPD reactivity (i.e. a high threshold of reactivity), gloves with poorer performance could be used. However, the fact that many hairdressers use the same gloves all day long implies that the use of gloves with poorer performance will only lead to an 'in vivo usage test' for the patients: they will more or less continuously be exposed to the allergens. It is therefore concluded that it is of utmost importance for the best type of glove to be used, and for hairdressers to use the gloves in the correct way, as has been previously shown in educational programmes.

Acknowledgements

The authors wish to thank Dr Flora Sonnsman and Professor Swen Malte John for advice and help in acquiring the needed gloves from Germany, and Lena Persson and Lotta Thorsson for skilful assistance during the study.

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