Permeability of Hair Dye Compounds 
\(p\)-Phenylenediamine, Toluene-2,5-Diaminesulfate and Resorcinol through Protective Gloves in Hairdressing

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Received 20 December 2006; in final form 27 April 2007; published online 25 June 2007

Dermal exposure to skin irritants and contact allergens is frequent in hairdressing. Hair dyeing is popular today and involves exposure to highly potent contact allergens, such as \(p\)-phenylenediamine (PPD). Use of protective gloves to prevent contact with skin-damaging substances is essential. The aim of the present study was to determine the resistance to permeation by PPD, toluene-2,5-diaminesulfate (TDS) and resorcinol (RES) through protective gloves used in hairdressing in Sweden. The permeation of PPD, TDS and RES through four types of protective gloves made of natural rubber latex (NRL), polyvinylchloride (PVC), nitrile rubber (NR) and polyethylene (PE) was tested using the American Society for Testing and Materials (1-inch) test cell. Exposure solutions were 5\% PPD (w/v), 0.75\% TDS and 10\% RES in borate buffer with 0.2 M ascorbic acid. The cumulative breakthrough, the so-called ‘time-lag breakthrough’ (Lag-BT), and permeation rate were determined for each substance and glove. For the NRL glove, the permeated amounts were below the analytical detection levels for all the tested substances. The NR glove was permeated only by RES, with a Lag-BT of 183 min. The PE glove was the thinnest glove and had a Lag-BT of 32 min for PPD; however, the steady-state permeation rate was only 0.031 nmol cm\(^{-2}\) min\(^{-1}\). The PVC glove gave the lowest protection against PPD and RES. TDS did not permeate any of the tested gloves. All the tested gloves were disposable, and all need to be changed often and disposed of after use. In conclusion, if properly used, all the tested gloves give considerable protection against permeation of PPD, TDS and RES.

\textbf{Keywords:} \textit{aromatic amines; hair colour; hairdresser; permanent hair dye; skin}

INTRODUCTION

Hairdressers have an increased risk of developing occupational skin diseases such as hand eczema (Lind \textit{et al.}, 2007). Hairdressing involves frequent wet work and repeated manual contact with many skin irritants and sensitizers. Hair dyeing is one of the most common hair treatments today, with permanent oxidative hair dyes being the most common type on the world market (Corbett, 1991). Permanent hair dyes contain compounds that can cause contact allergy, such as \(p\)-phenylenediamine (PPD), toluene-2,5-diamine (TDA) or its sulphate toluene-2,5-diaminesulfate (TDS) and resorcinol (RES). The total annual consumption of PPD, TDA and RES in the form of hair dyes in Europe amounted to 270 tons during 2002, according to the European Cosmetic, Toiletry and Perfumery Association (Søsted \textit{et al.}, 2004a). A questionnaire investigation carried out by the Medical Product Agency in Sweden among 30 registered suppliers of hair dyes showed that of 73 products on the Swedish market in 1999, 30\% contained PPD and 74\% contained TDA (Wahlberg \textit{et al.}, 2002). Several clinical studies show that hairdressers run a high risk of developing occupational allergic contact dermatitis. PPD and TDA (or TDS) are the most common agents responsible for allergic reactions, between 17 and 58\% of patch-tested hairdressers showing...
positive reactions to PPD in different studies and 14–25% to TDA or TDS (Armstrong et al., 1999; Iorizzo et al., 2002; Nettis et al., 2003; Uter et al., 2003). RES is also a contact allergen and is included in hairdressing patch test series (van der Walle, 2000). Apart from the local effect, systemic diseases may follow exposure to permanent hair dyes. Studies indicate an increased risk of bladder carcinoma both among hairdressers and among women who use permanent hair dyes (Gago-Dominguez et al., 2001; Gago-Dominguez et al., 2003; Andrew et al., 2004). According to the European Economic Community Cosmetics Directive, PPD is allowed in hair dye products, with a concentration limit of 6%; the TDA/TDS limit is 10%. During hair dyeing, the hair dye creams are mixed with hydrogen peroxide (3–12%), usually 50/50 so the maximum concentration in the hair dye mixtures that are used is 3% PPD and 5% TDA/TDS. Standard hair dye formulations marketed in industrialized countries today contain a maximum of 2% PPD in 100 ml dye solution (Nohynek et al., 2004).

In a previous investigation performed in hairdressing salons in Sweden, the contents of hair dye compounds in 22 hair dye mixtures were analysed and found to contain 0.004–0.250% PPD, 0.019–0.447% TDS and 0.001–0.271% RES (w/w) (Lind et al., 2005).

A recent study showed that hairdressers are exposed to compounds in permanent hair dyes during application of the dyes and also, while cutting newly dyed hair (Lind et al., 2005). The exposure loadings found were at levels at which there are a risk of sensitization and/or elicitation of an existing contact allergy. The study showed that the use of protective gloves does not always reduce exposure. This could be due to permeation through the glove material or penetration through holes or welded seams in the glove. Another reason may be that exposure occurred from contact with contaminated areas or handling hair dyes before the gloves were put on.

Standardized tests exist to evaluate penetration (leakage) and permeation of protective gloves. Theoretically, permeation can be described as a molecular process in three stages, the first being sorption on the surface of the material, the second diffusion through the material and the third desorption on the other surface of the material (Mellström et al., 2005). Diffusion, according to Fick’s first law, can be described as a function, where the permeation rate depends on the concentration of the chemical and the thickness of the material, and where

\[ P_s = L \times C = D \times C \times K / x, \]

\[ P_s \] = the rate of transfer per unit area at steady state (nmol cm\(^{-2}\) min\(^{-1}\)),

\[ C \] = the concentration in the vehicle (nmol cm\(^{-3}\))

\[ K \] = the partition coefficient vehicle per glove membrane,

\[ L \] = the permeation coefficient (cm min\(^{-1}\)),

\[ x \] = the distance within the material, i.e. the thickness of the material (cm) and

\[ D \] = the diffusion coefficient, a proportionality constant which is dependent on physico-chemical properties of the permeate (cm\(^2\) min\(^{-1}\)).

The protective effect of a glove does not only depend on the material formulation but also depend on other factors like manufacturing process, material combinations and material thickness. The permeation rate through of the same material may vary between brands and batches. In hairdressing, the most commonly used disposable glove materials are natural rubber latex (NRL) and polyvinylchloride (PVC). Other glove materials found on the market and used by hairdressers are polyethylene (PE) and nitrile rubber (NR).

The aim of this study was to determine the resistance to permeation by PPD, TDS and RES through protective gloves used in hairdressing using a permeation test method.

**MATERIALS AND METHODS**

**Gloves**

Four different disposable, non-powdered protective gloves used by hairdressers in Sweden at the time of the investigation were tested for permeation of PPD, TDS and RES. The gloves were provided by the hairdressers’ trade association in Sweden. Details of the gloves are presented in Table 1. Samples in the shape of a triangle (base 8 cm and sides 9.5 cm) were cut from the palm and back of unused gloves, the base of the triangle situated just beneath the base of the thumb. The exposed membrane area (given by the design of the test cell) was 4.15 cm\(^2\). The thickness of the material was measured according to ISO 4648 (23529-2004) at five points, one central and four peripheral, using a spring-loaded calliper (Oditest; Kroeplin, Schlüchtern, Germany). The mean ± standard deviation (SD) was calculated for each material.

**Chemicals**

The following chemicals were used: PPD [CAS 106-50-3] (97%), TDS [CAS 615-50-9] (97%), and RES [CAS 108-46-3] (99%), all from Lancaster Synthesis, Lancaster, UK. l(+)-ascorbic acid p.a. [CAS 50-81-7] (>99.7%), sodium tetraborate decahydrate
[CAS 1303-96-4] (>99%), methanol [LiChrosolv, high-performance liquid chromatography (HPLC) grade >99.8%] and hydrochloric acid (37%) were obtained from Merck KGaA (Darmstadt, Germany). Pure water (15 MΩ cm−1 quality) was obtained using a PURELAB Option R7 system (Elga, Bucks, UK). Buffer solution, pH 8.0, was prepared by mixing 440 ml 0.1 N hydrochloric acid and 560 ml 0.05 M sodium tetraborate. Standard solutions of PPD, TDS and RES were prepared in buffer and ascorbic acid was added as an antioxidant to a final concentration of 0.2 M.

**Standard test method**

Permeation testing of the chemicals was done using the American Society for Testing and Materials (ASTM) (1-inch) test cell (Mellström et al., 2005). The 2-inch ASTM cell is used in the ASTM F 739 standard (ASTM, 2003). A comparison between the 1- and 2-inch ASTM cell has been made, and the two cells were found to be equivalent (Vahdat, 1988). The tests were performed at room temperature (23.6 ± 2°C). The cell used is a two-compartment chemical permeation cell made of glass, internal diameter 2.5 cm. The material to be tested was placed vertically without tension as a barrier between the two compartments, the gloves’ normal outside surface facing the exposure compartment. The exposure compartment contained the exposure solution, and the collecting compartment contained 0.2 M ascorbic acid in buffer solution as a collecting medium. The total volume of the collecting medium was 22 ml. The collecting medium was circulated in a closed-loop system with a flow rate of 5 ml min−1 using an HPLC pump (JASCO PU-980 one-piston pump; JASCO, Tokyo, Japan) and continuously mixed with a magnetic stirring bar in the collecting compartment. Exposure solutions were 5% PPD (w/v), 0.75% TDS and 10% RES in buffer with 0.2 M ascorbic acid. Each glove was tested five times for PPD and RES and three times for TDS.

**Sampling**

Aliquots of 200 μl were taken from the collecting medium at 15-min intervals and placed in vials prior to HPLC analysis. Two hundred microlitres of 0.2 M ascorbic acid in buffer solution was added to the collecting chamber to make up the volume of the collecting medium. A sample of the collecting medium was taken just before the start of the test to check for possible contamination.

**Chemical analysis**

The samples were analysed using an HPLC instrument consisting of a pump, solvent degasser, auto sampler, column oven and diode array detector (Merck Hitachi LaChrom D-7000 HPLC; Merck KGaA). The column was a Merck LiChrospher RP 60 Select B, 250 × 4 mm, 5 μm particle size. The sample volume was 10 μl. The column temperature was 30°C. External standards of each of the tested chemicals were used for calculating the concentration. The HPLC analysis method has been described elsewhere in detail (Lind et al., 2004). The wavelength used for detection was 201 nm. The detection limit was 0.2 nmol ml−1. Minimum detectable mass permeated was 1.06 nmol cm−2 (which corresponds to 0.24 μg cm−2 for TDS and 0.12 μg cm−2 for PPD and RES).

**Calculation of permeation rate, lag breakthrough time and permeation coefficient**

The permeation rate initially is relatively low, but then it increases and at steady state becomes virtually constant per time unit, that is, at steady state the cumulated amount of chemical/area of the material specimen contacted (Q) can be described as $Q = P_s \times t$, where $Q$ equals to $C \times V/A$; $C$ is the concentration of the test chemical in collection medium, $V$ is the volume of the collection medium and $A$ is the area of the exposed membrane. This formula can be derived from Fick’s law as is shown by Schwope et al. (1988). The permeation rate at steady state ($P_s$) was calculated as the slope of the curve achieved by plotting the cumulated amount of chemical in the collecting medium against time. The breakthrough time is the elapsed time (in minutes) between the initial application of a test chemical in the exposure compartment and its subsequent presence on the other side of the material. The cumulative breakthrough, the so-called ‘time-lag breakthrough (Lag-BT)’, was calculated as the intercept of the linear portion at $P_s$ with the x-axis (Fig. 1). The Lag-BT is independent of the detection limit of the analytical method. This is in contrast to breakthrough time.

### Table 1. Description of the tested protective gloves

<table>
<thead>
<tr>
<th>Glove</th>
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<th>Manufacturer</th>
<th>Thickness (mm)</th>
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<tr>
<td>A</td>
<td>PVC (powder free)</td>
<td>Evercare Soft</td>
<td>SelefaTrade AB</td>
<td>0.12 ± 0.008</td>
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<td>B</td>
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<td>Touch N Tuff</td>
<td>Ansell Protective Products</td>
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<td>C</td>
<td>NRL (powder free)</td>
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<td>Semperit Technische Produkte GmbH &amp; Co KG</td>
<td>0.20 ± 0.008</td>
</tr>
<tr>
<td>D</td>
<td>PE/copolymer (powder free)</td>
<td>Unknown</td>
<td>Unknown</td>
<td>0.02 ± 0.01</td>
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*x* #1988). The tests were performed at room temperature (23.6 ± 2°C). The cell used is a two-compartment chemical permeation cell made of glass, internal diameter 2.5 cm. The material to be tested was placed vertically without tension as a barrier between the two compartments, the gloves’ normal outside surface facing the exposure compartment. The exposure compartment contained the exposure solution, and the collecting compartment contained 0.2 M ascorbic acid in buffer solution as a collecting medium. The total volume of the collecting medium was 22 ml. The collecting medium was circulated in a closed-loop system with a flow rate of 5 ml min−1 using an HPLC pump (JASCO PU-980 one-piston pump; JASCO, Tokyo, Japan) and continuously mixed with a magnetic stirring bar in the collecting compartment. Exposure solutions were 5% PPD (w/v), 0.75% TDS and 10% RES in buffer with 0.2 M ascorbic acid. Each glove was tested five times for PPD and RES and three times for TDS.

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which decreases with a decreasing detection limit. The test was ended after 4 h, unless in cases where \( P_s \) was achieved earlier. The estimated amount permeated of the test chemical through the glove material after \( t \) minutes was calculated as \( Q = (t - t_{\text{Lag-BT}}) \times P_s \). The permeation coefficient \( (L) \) was calculated as \( L = P_s/C_e \), where \( C_e \) is the concentration of the test chemical in the exposure compartment.

**RESULTS**

The Lag-BT and \( P_s \) for RES, PPD and TDS are summarized in Table 2. Two typical permeation curves are shown in Fig. 1.

**Time-lag breakthrough**

Glove C (NRL) showed no permeation for any of the tested substances. Glove B (NR) had a Lag-BT of 183 min for RES but was not permeated by PPD. Glove A (PVC) had a Lag-BT of \( \sim 90 \) min for PPD and RES while glove D (PE) had a Lag-BT of 32 min for PPD and 119 min for RES. None of the tested gloves was permeated by TDS.

**Steady-state permeation rate**

Glove D (PE) had the lowest \( P_s \) for RES and PPD. The highest \( P_s \) was seen for glove A (PVC) as well as for glove B (NR) with RES.

![Fig. 1. Permeation curves for RES. Glove A (PVC) on the left and glove B (NR) on the right. The Lag-BT is achieved as the intercept crosses the x-axis.](image)

**Permeation coefficients**

The permeation coefficients are shown in Table 3. The permeation coefficient was lower for the PE glove both for RES and for PPD.

**Estimated amount permeated**

The estimated amount permeated after 2 and 4 h is presented in Table 4. The estimated amount permeated after 4 h was highest for glove A, both for RES and for PPD.

**DISCUSSION**

The permeation of PPD, TDS and RES through protective gloves commonly used by hairdressers was tested using a standardized procedure. The gloves tested were made of PVC, NR, NRL and PE. All the tested gloves had a Lag-BT of >1 h, with the exception of the PE glove that had a Lag-BT of 32 min for PPD; however, the permeation rate for the PE glove was very low. During 4 h, none of the tested substances permeated the NRL glove. The NR glove was the second best, with only RES being able to permeate this glove, with a Lag-BT of \( \sim 3 \) h. The PVC glove gave the lowest protection against PPD and RES. The PE glove was the thinnest glove but the permeation rate through this glove was very low. According to the ASTM F 739 standard, for a closed-loop test the normalized breakthrough detection time should be reported. This is defined as the time when the mass of chemical permeated reaches 0.25 \( \mu \text{g cm}^{-2} \) (2.3 nmol cm\(^{-2}\) for RES and PPD and 1.1 nmol cm\(^{-2}\) for TDS). In the European Standard EN 374-3:2003 the breakthrough of a chemical (or mixture) is deemed to have occurred when the permeation rate of each individual component reaches a rate of 1 \( \mu \text{g cm}^{-2} \) min (9 nmol cm\(^{-2}\) min\(^{-1}\) for RES and PPD and 4.5 nmol cm\(^{-2}\) min\(^{-1}\) for TDS). For all tested gloves, the steady-state permeation rate was <9 nmol cm\(^{-2}\) min\(^{-1}\) (1 \( \mu \text{g cm}^{-2} \) min). This implies that for none of the tested combinations of chemicals and gloves, permeation would have been considered to have occurred according to the EN 374-3. This is a disadvantage of the standard test method since

![Table 2. Arithmetic means and SD for Lag-BT (in minutes) and permeation rates at steady state \( (P_s) \) (nmol cm\(^{-2}\) min\(^{-1}\))](image)

<table>
<thead>
<tr>
<th>Glove</th>
<th>RES</th>
<th>Lag-BT (min)</th>
<th>( P_s ) (nmol cm(^{-2}) min(^{-1}))</th>
<th>PPD</th>
<th>Lag-BT (min)</th>
<th>( P_s ) (nmol cm(^{-2}) min(^{-1}))</th>
<th>TDS</th>
<th>Lag-BT (min)</th>
<th>( P_s ) (nmol cm(^{-2}) min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>90 ± 3.9, n = 5</td>
<td>3.6 ± 0.7, n = 5</td>
<td>86 ± 4.6, n = 5</td>
<td>0.88 ± 0.1, n = 5</td>
<td>&gt;240, n = 3</td>
<td>n.b.</td>
<td>&gt;240, n = 3</td>
<td>n.b.</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>183 ± 2.1, n = 5</td>
<td>3.9 ± 0.6, n = 5</td>
<td>&gt;240, n = 5</td>
<td>n.b.</td>
<td>&gt;240, n = 3</td>
<td>n.b.</td>
<td>&gt;240, n = 3</td>
<td>n.b.</td>
</tr>
<tr>
<td>C</td>
<td>&gt;240, n = 5</td>
<td>n.b.</td>
<td>&gt;240, n = 5</td>
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<td>&gt;240, n = 3</td>
<td>n.b.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>119 ± 6.8, n = 5(^*)</td>
<td>0.0079 ± 0.0006, n = 5(^*)</td>
<td>32 ± 23, n = 4</td>
<td>0.031 ± 0.0031, n = 4</td>
<td>240, n = 3</td>
<td>n.b.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n.b., no breakthrough detected within 4 hours.

\(^*\)Permeates in small quantities (near the detection limit), very low permeation rate.
allergic individuals may react to very low amounts of contact allergens and this low permeation may not be accounted for in the test.

Test results such as these can be affected by several parameters (Mellström et al., 1994), such as flow rate and mixing, cell size and design, using an open- or closed-loop system, choice of test cell, and temperature, as well as the thickness and formulation of the tested material. Several authors have studied permeation rate at different temperatures (Stampfer et al., 1984; Vahdat and Bush, 1989; Zellers and Sulewski, 1993). Vahdat and Bush showed that both breakthrough time and permeation rate vary exponentially with temperature, but breakthrough time is more dependent on temperature changes. In our experiments, we have not been able to control the temperature in the collecting medium. This is a potential source of error; still, the temperature variation was small and the collecting medium. This is a potential source of error; still, the temperature variation was small and the collecting medium.
that it is not the gloves per se that are the problem. The materials withstand permeation well; however, the way gloves are used may well contribute to the exposure. Gloves are often put on too late (i.e. well into the treatment). Also, disposable gloves are reused, gloves are turned inside out and reused and protective gloves are sometimes used for several months or until they become torn. All the tested gloves gave protection for ≥30 min. Our recommendation is that the glove considered most comfortable by the user should be chosen. It is important, however, not to reuse disposable gloves and to wear gloves during all work tasks during which skin contact with hair dyes or other hair cosmetic products may occur. Since allergic reactions against NRL may be life threatening, gloves made from NR are recommended as the best choice for use in hairdressing. As an alternative, PVC gloves may be used if they are changed often and are not used for >1 h. The PE glove is thin and is quickly permeated; however, the permeated amounts are usually small.

In conclusion, all the tested gloves give considerable protection against permeation of PPD, TDS and RES provided they are properly used.

FUNDING

Acknowledgements—We would like to thank Jan Sollenberg for valuable assistance regarding HPLC analyses and manuscript preparation.

REFERENCES

