Permeation of Hair Dye Ingredients, *p*-Phenylenediamine and Aminophenol Isomers, through Protective Gloves

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Skin irritation and contact allergies are skin disorders common to hairdressers. The predominant oxidative hair dye components, such as *p*-phenylenediamine (PPD) and aminophenol isomers, can cause contact dermatitis. Use of protective gloves can prevent dermal contact with skin irritants. This study investigates the permeation behaviors of *p*-aminophenol (PAP), *m*-aminophenol (MAP), *o*-aminophenol (OAP) and PPD in single and mixed challenge solutions with disposable natural rubber latex (NRL) gloves, disposable polyvinylchloride (PVC) gloves and neoprene (NP) gloves. The challenge solutions were 4% PPD (w/v), 3% OAP (w/v), 2% PAP (w/v) and 2% MAP (w/v) in ethanol or 12% hydrogen peroxide solutions. The cocktail solutions of the four chemicals were also tested. An American Society for Testing and Materials type permeation cell, ethanol liquid collection and gas chromatography–flame ionization detection of samples taken from the collection medium every 10 min facilitated determination of breakthrough times (BTs), cumulative permeated masses and steady-state permeation rates (SSPRs). Experiments were 4 h long for the NRL and PVC gloves and 8 h for NP gloves. No chemicals tested broke through the NP gloves when exposed for 8 h. In the ethanol solution, PPD and OAP started breaking through the PVC gloves at 40 min. The SSPRs of PVC gloves were higher than those for NRL gloves in all challenge conditions for both single chemicals and mixtures. No tested chemicals in hydrogen peroxide solutions permeated the gloves during the 4-h tests. The chemical composition of the challenge solution was a main effecter of BTs and SSPRs for the NRL glove. For disposable PVC gloves, the main factors of BTs were molecular size [molar volume (MV)] and polarity (log Kow), and the primary factors of SSPRs were concentration, MV and log Kow. In conclusion, disposable NRL gloves and disposable PVC gloves should not be used repeatedly for handling the hair dye products. Hydrogen peroxide did not accelerate chemical breakthrough. The compositions of the challenge solutions and physical and chemical properties (MV and log Kow) affected permeation behaviors for different gloves.

Keywords: aminophenols; chemical-protective gloves; hair dyes; permeation; *p*-phenylenediamine

INTRODUCTION

The International Agency of Research on Cancer (IARC) estimated that the number of hairdressers and barbers worldwide in 1993 totaled several millions. Approximately, 35% of women and 10% of men in Europe, Japan and the USA have used hair colorants (IARC, 1993). Notably, hairdressers have an increased risk of occupational skin diseases due to exposure to skin irritants and sensitizers (Uter et al., 2003). Hair dyes are categorized by the coloring persistence, permanent hair dyes (oxidative hair dyes), semipermanent hair dyes and temporary hair dyes (IARC, 1993). Oxidative hair dyes contain primary intermediates and couplers. The primary intermediates are *p*-phenylenediamine (PPD), *o*-aminophenol (OAP), *p*-aminophenol (PAP) or *p*-toluenediamine, whereas the couplers are *m*-aminophenol (MAP), *m*-hydroxyphenol or resorcinol. When mixing intermediates and couplers, the primary intermediates initially react with hydrogen peroxide to form a diimine, and the diimine then reacts with couplers to form dinuclear, trinuclear or polynuclear structures. The small molecules (unreacted primary intermediates and couplers) diffuse into hair,
start coupling reactions and then become trapped in hair (Nohynek et al., 2004; Rastogi et al., 2001; Bolt and Golka, 2007). A skin exposure assessment study indicated that hairdressers were exposed to compounds in permanent hair dyes during dye application and when cutting newly dyed hair (Lind et al., 2005). Thus, the unreacted chemicals entrapped in hair increased the probabilities of contacting these chemicals.

Several epidemiological studies reported that occupational exposure to hair dyes caused an increased risk of bladder cancer for male hairdressers (IARC, 1993; Czene et al., 2003; Kogevinas et al., 2003; Gaerttner et al., 2004). Those who have worked as hairdressers or barbers for at least 10 years have a 5-fold increase in risk compared with individuals not exposed to hair dyes (Gago-Dominguez et al., 2001). Several recent meta-analyses of epidemiological studies concluded that insignificant causal association exists between a hair dye user and bladder cancer (Hunchareik and Kupelnick, 2005; Takkouche et al., 2005; Kogevinas et al., 2006; Lin et al., 2006; Kelsh et al., 2008).

Hairdressers typically come in contact with different hair products, such as hair dyes, during work (Iorizzo et al., 2002; Søsted et al., 2004, 2005). The sites of hair dye contact allergies for hairdressers are hands and forearms (Iorizzo et al., 2002; Søsted et al., 2002). A German study demonstrated that 73.1% of all hairdressers experienced occupational contact dermatitis, 89.3% of which had contact dermatitis on the hands (Uter et al., 2003). Uter’s group also reported a high prevalence of hairdressers’ contact allergies to PPD according to the data from Information Network of Departments of Dermatology (Uter et al., 2007).

Several studies have identified PPD and aminophenol isomers, which are the major components of commercial oxidative hair dye products (especially in Taiwan), as common allergens for hair dye (Wu et al., 1997). Varying degrees of chronic inflammation of the skin were observed in mice after contact with the oxidative hair dyes for a period of 20 months (Jacobs et al., 1984). Notably, PPD is one of the most potent contact allergens. Of the 29% of the persons who had adverse reactions to hair dyes and underwent patch tests, all were positive for allergic reaction to PPD (Søsted et al., 2002). Wearing appropriate protective gloves while contacting the hair dye to limit the exposure to allergic chemicals for hairdressers is recommended (Nixon et al., 2006). Nitrile rubber gloves have been tested for 5% PPD (w/v) and no breakthrough was detected at 4 h (Lind et al., 2007).

Permeation occurs when a chemical contacts the glove surface and diffuses through the glove material. The chemical molecules traveling through the glove material then contact skin. Several factors affect the ability of a given chemical to permeate through glove materials. The important factors are the glove material itself, additives used during glove manufacturing, the manufacturing process and glove material thickness. Permeation parameters primarily include breakthrough time (BT) and steady-state permeation rate (SSPR). These parameters indicate how long gloves provide reliable protection when contacting a particular chemical. These parameters are also critical references for selection and use of gloves. Therefore, evaluating the permeation parameters of chemical-protective clothing in contact with different chemicals is necessary.

The aims of this study were as follows: (i) determine the permeation parameters of PPD, PAP, MAP and OAP while challenging disposable natural rubber latex (NRL) gloves, disposable polyvinylchloride (PVC) gloves and neoprene (NP), gloves using the American Society for Testing and Materials (ASTM) F739 method, (ii) investigate the effect of hydrogen peroxide on hair dye permeation, (iii) recommend the appropriate protective gloves to wear when dealing with hair dye products and (iv) determine which factors affect the permeation behavior for these investigated gloves.

**MATERIALS AND METHODS**

**Gloves and chemicals**

Three gloves were subjected to permeation tests: disposable powder-free NRL gloves (DERMA-GRIP®, D1501-30, WRP Asia Pacific Sdn Bhd, Sepeng, Malaysia), disposable PVC gloves (9” PVC gloves, P0014-9, Carry Hi Technical Corpora-

tion, Taipei County, Taiwan) and NP gloves (Neo-

prene™, 29-865, Ansell, Red Bank, NJ, USA). The disposable NRL gloves and disposable PVC gloves are available at affordable prices and used widely by hairdressers. The NP gloves have good permeation resistance against ethanol and hydrogen peroxide solution according to the manufacturer’s test data.

Four hair dye ingredients were selected based on an up-to-date literature review and survey of common commercial hair dye products in Taiwan (Wu et al., 1997). The PPD (CAS: 106-50-3, GC/NT grade, purity ≥ 97.0%), PAP (CAS: 123-30-8, grade, purity ≥ 97%) and MAP (CAS: 519-27-5, technical grade, purity ≥ 98%) were from Fluka (Steinheim, Germany) and OAP (CAS: 95-55-6, technical grade, purity 99%) was also acquired from Fluka. Ethanol (CAS: 64-17-5, purity 98%) was also purchased from Aldrich, Munich, Germany. The internal standard, biphenyl (CAS: 92-52-4, GC grade, purity 98%) was obtained from Aldrich, Munich, Germany. The internal standard, biphenyl (CAS: 92-52-4, GC grade, purity 98%) was also purchased from Fluka. Ethanol (CAS: 64-17-5, purity 95%) was purchased from Taiwan Sugar Corporation (Tainan, Taiwan). The 35% hydrogen peroxide solution (CAS: 7722-84-1) was obtained from Riedel-de Haën (Hanover, Germany).

**Apparatus**

The ASTM-type I-PTC-600 permeation test cells were obtained from Pesce Lab Sales (Kennett...
Square, PA, USA). A micrometer screw gauge (SM-112 00-398-04, Teclock, Tokyo, Japan) was used to measure glove thickness at seven random points before and after each permeation experiment. A torque wrench (OP-60, Onpin, Taichung, Taiwan) ensured equal tightness of permeation cell nuts. An analytical electrical balance (XS 204, Mettler Toledo, Greifensee, Switzerland) was employed to weigh the glove samples before and after each permeation experiment.

Chemical analysis utilized an Agilent 7890A gas chromatograph with 7683B autoinjector module and flame ionization detector (GC–FID) (Santa Clara, CA, USA). The fused capillary column was a Restek Rtx®-35 30 m × 0.25 mm I.D. with 0.25-µm film (Bellefonte, PA, USA). The carrier gas, nitrogen, was 99.999% pure and had a flow rate of 0.8 ml min⁻¹ (Shengyi Gas, Taipei, Taiwan).

Methods

The permeation test was a modified closed-loop system of the ASTM F739-07 method (ASTM, 2007) and has been described elsewhere (Lin and Que Hee 1998a,b,c). In summary, the glove materials from the palm or back parts of the gloves were cut into circles (diameter 5 cm). The specimens were conditioned for 24 h in a desiccator at 21.6 ± 1.6% relative humidity at room temperature before permeation tests. The glove sample was held between the two Teflon gaskets of the permeation cell. Two glass chambers were inserted into the aluminum flanges, and the nuts were tightened using a torque wrench. The exposed area of the glove material was 4.2 cm². A volume of 15 ml ethanol was added as the collection medium and 15 ml of challenge solution was pipetted into the challenge chamber. Different challenge solutions were tested (Table 1). The concentrations of each test chemicals were decided according to the legislatively allowable maximum concentrations of Council of the European Communities, Taiwan Department of Health and US Food and Drug Administration. Ethanol was used as the dilution solution because of the solubilities of the four investigated chemicals. Hydrogen peroxide was a solvent to mix hair dye cream before application. It was used to simulate the real hair dye application situation in this study. Aliquots of 990 µl were taken at 10-min intervals from the collection side and then introduced into 1.5-ml brown vials. A 1-µl aliquot of the permeation sample was injected into the GC–FID via the autoinjector module. The same volume (990 µl) of fresh ethanol was replenished into the collection side immediately after each sampling to maintain the total volume of the collection medium at 15 ml during the entire permeation experiment. A discontinuous shaking was performed every 10 min, i.e., before taking the samples and after replenishing the collection medium. Experiment durations were 4 h for disposable gloves (NRL and PVC) and 8 h for the NP gloves. At the end of each permeation experiment, the permeation cell was disassembled to remove the glove sample. The glove specimen was then weighed and its thickness measured immediately after being patted dry using a Kimwipe. Permeation tests were performed at a thermostatic-controlled room with average temperature of 21 ± 1°C. All experiments were performed in triplicate.

Operating conditions for GC–FID were as follows: 280°C for the injector, 250°C for the detector; initial column temperature was 60°C for 2 min, increasing to 150°C at 15°C min⁻¹, held for 5 min and then increased to 280°C at 35°C min⁻¹ and then held for additional 2 min. The nitrogen carrier gas flow rate was 0.8 ml min⁻¹. All chemicals including internal standard eluted within 15 min (Fig. 1). Internal standard calibration curves were generated for each analysis day. The GC–FID linear ranges of the analytical method were 1–10 ng µl⁻¹ for OAP, 1.5–10 ng µl⁻¹ for PAP and PPD and 2–10 ng µl⁻¹ for MAP. The minimum detectable mass permeated for the four chemicals in the collection solution were 15 µg for OAP, 22.5 µg for PAP and PPD and 30 µg for MAP.

The BT was the elapsed time between initial application of a chemical in the exposure compartment and its subsequent presence on the other side of the material (ASTM, 2007). Theoretically, SSPR is determined when the increase in permeation becomes constant relative to time. The total permeated mass of each chemical in the collection medium was calculated from the mass in the 1-µl injection using the internal standard method corrected for the fraction injected and for the mass in the previous permeation samples. Cumulative permeated mass was

<table>
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<tr>
<th>Table 1. The compositions of the challenge solutions</th>
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<td><strong>Chemicals</strong></td>
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<td>Ethanol solution</td>
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<td>Single chemical (w/v%)</td>
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<td>Mixtures (w/v%)</td>
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<tr>
<td>12% Hydrogen peroxide solution</td>
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<tr>
<td>Single chemical (w/v%)</td>
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<td>Mixtures (w/v%)</td>
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*Mixtures contained the four test chemicals with the listed concentrations.*
plotted versus sampling time. The linear range of the cumulative permeated mass curve was utilized to calculate the SSPR by simple linear regression analysis, i.e. the slope of the linear range of the curve (Fig. 2). The SSPR is typically adjusted by the exposed area and expressed in the unit, $\mu g\ cm^{-2}\ min^{-1}$ as in equation (1):

$$\text{SSPR} = \frac{\text{Slope of steady stage} (\mu g\ min^{-1})}{\text{Exposed area} (cm^2)}$$

All statistic analyses were performed by the SPSS Version 14.0 (Chicago, IL, USA). To determine the significance of possible factors, the stepwise linear regression analysis was applied. The criterion for adding or deleting an independent variable was based on the $F$-statistic when the probability of $F$ to enter was $\leq 0.05$ or the probability of $F$ to remove was $\geq 0.1$. Statistical significance was set at $P \leq 0.05$.

**RESULTS**

**Glove physical change**

The average initial glove thicknesses of NRL and NP did not significantly change for all challenges at $P \leq 0.05$ (non-parametric Kolmogorov–Smirnov Z-test). The PVC shrank significantly and became inflexible after being exposed to the chemical ethanol challenge solutions. The weight changes of the test specimens, NRL and NP were $<3\%$. For test chemicals in ethanol solution permeation, the weight of PVC decreased 7.16\%. The PVC glove thicknesses and weights decreased much while challenged with the chemical ethanol solutions.

**BT and SSPR**

Table 2 lists the ranges of BTs and SSPRs of single and mixed chemicals through three gloves from the triplicate experiments. Among all exposures, the earliest breakthrough was at 40 min for OAP and PPD in the ethanol solutions while challenging the PVC gloves. The BTs of the NRL glove materials were longer than those of PVC gloves for the four chemicals in ethanol solutions. For both NRL and PVC gloves, longer BTs for chemicals in hydrogen peroxide solutions were observed while compared to those in ethanol solutions. In all hydrogen peroxide solutions, only OAP in the cocktail solutions broke through at 140–210 min when challenging the PVC gloves. For the single chemical challenge solutions, PPD had the fastest SSPR for both NRL gloves and PVC gloves. The SSPRs of PVC gloves were higher than those of NRL gloves in all challenge conditions for both single chemicals and mixtures. The PAP did not breakthrough NRL gloves within 4 h. The NP
gloves were not permeated by any tested substances during 8-h exposure.

**Cumulative permeated mass**

Table 3 lists the total cumulative permeated masses for the NRL and PVC gloves. For NRL gloves, the total cumulative permeated masses of the three breakthrough chemicals in ethanol solutions were, in a decreasing order, PPD > OAP > MAP. For PVC gloves, the total cumulative permeated masses of the four breakthrough chemicals in ethanol solutions were, in a decreasing order, PPD > OAP > MAP > PAP. The cumulative permeated masses of PVC gloves were larger than those of NRL gloves. For mixture solutions, the only breakthrough chemical was OAP for PVC gloves, with a total cumulative mass of 31.05 µg.

**Predictor analyses of the permeation parameters**

Perkins (1990) demonstrated that the chemical concentration (C), molar volume (MV) and octanol–water partition coefficient (logKow) affect permeation behavior. All these parameters, C, MV and logKow, were tested as independent variables to explain the results for BTs and SSPRs from different challenge conditions. Table 4 shows the statistically significant predictors of permeation parameters with the square of the correlation coefficient (r²) and
probability $P$-values for two tested gloves in ethanol solutions. For NRL gloves, six significant correlations were obtained. The followings were regression solutions. For NRL gloves, six significant correlations for BT and SSPR were significant. For PVC gloves, only significant correlation for BT was obtained. Thus, hairdressers must change NRL gloves once they finish the dyeing process, which usually takes 1 h. The PVC gloves had a BT $> 60$ min. Hairdressers should be informed of this short BT and change gloves frequently.

The ability of three gloves to withstand four ingredients common in hair dyes was tested by exposing the gloves to individual chemicals and mixtures of the chemicals in ethanol and hydrogen peroxide solutions. No breakthrough in an 8-h test indicates potentially complete protection should hairdressers have a continuous 8-h shift. All NP glove withstood exposure for 8 h. All NRL gloves had a BT $> 60$ min. Thus, the hairdressers must change NRL gloves once they finish the dyeing process, which usually takes 1 h. The PVC gloves had a BT $> 60$ min. Hairdressers should be informed of this short BT and change gloves frequently. In terms of the BTs and SSPRs, the NRL gloves provide better protection than PVC gloves for four tested chemicals. Lind et al. (2007) found that NRL gloves provided better protection than PVC for gloves when challenged with 5% PPD, which is similar to experimental results obtained by this study. However, glove thicknesses varied even when gloves were of the same type.

Two coefficients, diffusion coefficient ($D$) and permeability coefficient ($P$), reveal the protecting effect of glove material independent of the thickness. The $D$ (cm$^2$ min$^{-1}$) can be calculated from $D = L^2/6t_l$, where $L$ is the thickness of glove material and $t_l$ is the lag time which is given by the time–axis intercept from the extrapolation of the steady-state portion of the cumulative permeation curve (Fig. 2) (Perkins, 1990). In this study, the Ds of PPD were $4.02 \times 10^{-7}$ for NRL, and $4.04 \times 10^{-7}$ for PVC. The Ds of OAP were $5.07 \times 10^{-7}$ for NRL and $4.14 \times 10^{-7}$ for PVC. The $P$ ($\mu$g cm$^{-1}$ min$^{-1}$) is the product of SSPR and $L$ (Chao et al., 2007). The Ps of PPD were $3.78 \times 10^{-4}$ for NRL and $5.09 \times 10^{-4}$ for PVC. The Ps of OAP were $2.32 \times 10^{-4}$ for NRL and $3.58 \times 10^{-4}$ for PVC. NRL gloves should be more efficient in preventing the skin exposure to the hair dye chemicals while comparing to PVC gloves due to the slightly small values of these two coefficients.
These experimental data are the first published that show the hydrogen peroxide did not accelerate the hair dye permeation through gloves. No data are available in literature for the permeation through gloves of mixtures of four chemicals and chemicals in hydrogen peroxide solutions. Only OAP broke through the PVC glove after 2 h (BT = 140–210 min). Monticello and Gaber (1999) reported that PVC gloves 0.11 mm thick provided ≥30 min of protection to 7.5% hydrogen peroxide. The NRL gloves (0.42 mm thick) and NP gloves (0.38 mm thick) provided excellent protection throughout the 8-h tests. The thickness of PVC gloves of this study was same as that in the study by Monticello and Gaber, even though the gloves were obtained from different manufacturers. Hydrogen peroxide may have permeated the PVC gloves during the tests if the permeation behaviors were similar to that of the PVC gloves in the study by Monticello and Gaber. This study did not investigate the permeation of hydrogen peroxide and no measurements were performed. The co-solvent effect of 12% hydrogen peroxide was not identified based on study findings. Lind et al. (2004) confirmed the oxidation process for 12% hydrogen peroxide by mixing PPD and MAP, 2.5% of PPD and 17% of MAP remained after 60 min. As the concentrations of the challenge chemicals were identified as a significant predictor of permeation behavior (Table 4), no major breakthrough was observed due to the decrease of the chemical concentrations of the challenge solutions. This explains why no significant permeation was observed when chemicals were dissolved in the hydrogen peroxide solution.

Table 4 shows the statistically significant predictors of permeation parameters for the NRL and PVC gloves. The chemical concentrations in the challenge solutions (C) significantly affected the BT and SSPR for the NRL gloves, but only significantly affected SSPR for PVC gloves. This finding is also based on the BTs (Table 2). The challenge concentrations of PPD were 4% in the single chemical and 1% in cocktail solutions; the range of BTs was 40–60 min for the 4% PPD and 70–80 min for the 1% PPD. A similar phenomenon existed for OAP. An increased challenge concentration of a particular chemical typically yielded an increased cumulative permeated mass (Table 3). For instance, the initial PPD concentration in the single chemical challenge solution was four times that in the mixture challenge solution (4 versus 1%) and the means of the total cumulative permeated masses of these two challenge solutions were proportionally related. The proportional relationships were 154.43 versus 41.15 µg for the NRL glove tests and 507.2 versus 130.96 µg for PVC glove tests. These experimental data demonstrated that the initial mass of a chemical in challenge solution influences permeated mass.

The MV was a significant factor for BTs for the glove materials, NRL and PVC. The small molecules with small MV values move rapidly and tend to breakthrough the glove quickly according to the chromatographic model (Que Hee, 1996). This rapid breakthrough resulted in MV being a positive factor in predicting BT. Alone a similar line, MV affected the SSPR negatively, implying that large molecules move slowly within glove material, resulting in a low SSPR.

The logKow, a surrogate of the chemical polarity, was also a significant factor affecting BT and SSPR. According to the liquid–liquid partition model, a chemical permeates a glove material rapidly when the polarities of the challenge chemical and the polymer (glove material) are similar (Que Hee, 1996). The logKow was positively correlated with BT and negatively correlated with SSPR in the PVC glove tests. Notably, PPD, a hydrophile chemical with a negative logKow value, broke through fast at a high permeation rate when the PVC acted as a relative polar solvent. The significance of polarity was not observed for the NRL glove implying that the glove material itself has a significant role in permeation behavior. When all possible factors were involved, the significance of certain factors was decreased by other factors. To extrapolate these results to different challenge combinations should be conservative due to the limited sample size.

Evans et al. indicated that the temperature differential between the inside and outside of gloves adversely affected chemical BT and the permeation rate of a solvent passing through the glove material (Evans et al., 2001). Thus, when dying hair, the hair dye permeation process occurred at the temperature higher than this test temperature as hairdressers wear gloves. The temperature of the glove inside surface should rise rapidly when contacting the hairdresser’s skin. The BTs and permeation rates in practice shall be underestimated by the current experimental settings.

Commercial hair dyes are formulated using dozens of ingredients and varied for different colors and manufacturers. Commercial hair dyes are typically creams; however, challenge solutions of this study were liquid. Ethanol was able to shorten the BT of 2-hydroxyethyl methacrylate for PVC gloves (Andreasson et al., 2003). Ethanol therefore interferes with the permeation behavior of PVC. The essential permeation parameters of different glove materials reported comprise a reliable reference for glove selection.

In conclusion, disposable NRL gloves and disposable PVC gloves cannot be used repeatedly when in contact with hair dyes. NP gloves are safe for at least 8 h. Low concentrations of chemical in challenge solution delayed BT and decreased the SSPR. The compositions of challenge solutions and physical and
chemical properties (MV and log\(K_{ow}\)) affected the ability of different gloves to withstand permeation. Hydrogen peroxide did not accelerate the chemical BT.

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