P-phenylenediamine
Bijkersma-Pot, Laura

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Penetration and haptenation of \( p \)-phenylenediamine

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Abstract

Although p-phenylenediamine (PPD) has been recognized as an extreme sensitizer for many years, the exact mechanism of sensitization has not been elucidated yet. Penetration and the ability to bind to proteins are the first two hurdles that an allergen has to overcome to be able to sensitize. This review is an overview of papers regarding PPD penetration through skin (analogues) and papers studying the amino acids that are targeted by PPD. To complete this review, the auto-oxidation as well as N-acetylation steps involved in PPD metabolism are described. In summary, under normal hair dyeing exposure conditions, less than 1% of the applied PPD dose penetrates the skin. The majority (>80%) of PPD that penetrates will be converted into the detoxification products monoacetyl-PPD and diacetyl-PPD by the N-acetyltransferase enzymes. The small amount of PPD that does not become N-acetylated is susceptible to auto-oxidation reactions, yielding protein reactive PPD derivatives. These derivatives may bind to specific amino acid residues and some of the formed adducts might be the complexes responsible for sensitization. However, true in vivo evidence is lacking and further research to unravel the definite mechanism of sensitization is needed.
Introduction

*p*-Phenylenediamine (PPD) is an aromatic amine known for its extreme sensitizing potency, and may cause severe allergic contact dermatitis. It is predominantly used as a precursor in oxidative hair dyes, but it can also be found in so-called ‘temporary henna tattoos’, despite the prohibition of this application in Europe and the United States.\(^1\) Hair dyeing - in both consumer and occupational settings - has been shown to be a common cause of PPD sensitization.\(^3\) Whereas allergic contact dermatitis in consumers gives rise to erythema and oedema (sometimes severe) of the face, eyelids, scalp, and neck, the hands are most affected in hairdressers.\(^4\) Although allergic contact dermatitis caused by PPD has been studied extensively, the exact pathways of sensitization and elicitation are not yet completely unravelled. Nevertheless, as is generally known, in order to induce a T cell-mediated immune response, a chemical needs to undergo several steps. Briefly, a chemical has to penetrate the stratum corneum to reach the living epidermis. Here, the low molecular weight chemical, the hapten, will haptenate (i.e. be bound to) cutaneous protein. The hapten-protein complex will be formed inside the cell or be taken up and subsequently processed by antigen-presenting cells of the epidermis or dermis. These cells become activated and migrate to regional lymph nodes, where they may encounter and activate allergen-specific naïve T cells. Expanded progeny of these allergen-specific T cells, which are then turned into effector and memory T cells, will recirculate and migrate to the skin. On re-exposure to the initial allergen, the above mentioned process will recur, but now memory T cells can immediately release cytokines and chemokines, leading to the clinical features of allergic contact dermatitis. These general principles have been summarized extensively by Rustemeyer et al.\(^5\)

Although PPD is a common contact allergen, it has some special, specific properties, which make it such an interesting allergen. This article will focus on PPD-specific penetration and haptenation processes, that is, events prior to the activation of antigen-presenting cells and subsequent T cell stimulation. Although the latter two steps are also essential in sensitization, they are beyond the scope of this review. Regarding penetration, an overview of the amount of PPD that becomes available to affect the immune system will be given, whereas for haptenation, the targeted amino acids will be shown.

Penetration

A chemical that comes into contact with the human skin has to penetrate the skin barrier in sufficient quantities to be able to perform its immunogenic task.\(^6\) The stratum corneum plays the largest role in the physical barrier function of the skin.\(^7\) This is confirmed by studies on ‘tape stripping’, wherein the removal of the stratum corneum leads to an enormous increase in ab-
sorption of chemicals. 8 To a lesser extent, the epidermis also contributes to the barrier function by means of its desmosomal, junctional and cytoskeleton structures. 7 For penetration of the highly protective stratum corneum barrier, several conditions have to be met. Molecular size has been regarded as one of the most important determinants. Bos and Meinardi argued that the molecular weight (MW) of a chemical must be <500 to allow skin absorption, because larger molecules are uncommon as contact sensitizers and the most commonly used agents in dermatotherapy and transdermal drug-delivery systems have MWs <500. 6 Although low-MW chemicals penetrate more easily, larger molecules like proteins have been shown to be able to pass the stratum corneum. 9,10 Next to molecular size, lipophilicity - often expressed as a partition coefficient (log \( P_{\text{octanol/water}} \)) - and non-polarity are considered to be important physicochemical factors positively influencing the percutaneous absorption. 11-13 Nevertheless, hydrophilic, polar substances are also able to penetrate the skin. Theoretically, chemicals can penetrate the epidermal barrier via two distinct pathways: the transepidermal route, whereby the chemical penetrates intercellularly or intracellularly; or via diffusion along sweat glands and pilo-sebaceous follicles, the so called ‘shunt pathway’ or follicular penetration. Whereas the extent of contribution to transdermal delivery of the latter pathway is discussed, 14,15 and it is proposed that chemicals use several pathways simultaneously, 16 the intercellular pathway is generally considered to be the predominant route of penetration. 17-20 The intercellular matrix surrounding the corneocytes consists of lipid bilayers, which are organised in such a way that both lipophilic and hydrophilic domains, sometimes called ‘hydrophilic pores’, are formed, allowing the penetration of nonpolar and polar molecules respectively. 21-23 Less evidence is available for intracellular penetration, 13,24 although corneocytes may provide a hydrophilic pathway, owing to their protein content. Besides a chemical’s physicochemical parameters, several other factors are important in determining skin penetration. Exogenous factors such as extensive exposure to detergents and physical damage (e.g. irradiation or abrasion) impair the skin, and this may lead to irritation or even irritant contact dermatitis, 25 which, in turn, has been demonstrated to be important in development of allergic contact dermatitis. 26 On the other hand, pre-existing inflammatory skin conditions, 27 atopy 28 and loss-of-function null mutations in the gene encoding the skin barrier protein filaggrin (FLG) are endogenous factors known to affect the skin barrier. However, the relationship between FLG mutations and contact sensitization is not straightforward, as exposure may represent the major risk factor, as suggested for nickel sensitization. 29 Furthermore, as summarized by Berard et al., protein binding, hydration level, anatomical differences, occlusion, age and the vehicle used may influence overall skin penetration. 9 Although even small amounts of allergen may be sufficient to start the sensitization cascade, penetration remains the prerequisite for interaction with the cutaneous immunological cells. In the following paragraphs, the quantitative penetration of PPD through the skin will be discussed. In order to place these values in perspective, the results of penetration studies on other aromatic amines - toluene-2,5-diamine [\( \text{p-toluenediamine (PTD)} \)] and \( \text{p-aminophenol} \)
- are provided. Importantly, reported differences of three-fold to 11-fold \(^{30}\) fall in the commonly accepted range of interindividual differences in humans.

**PPD specific penetration under typical exposure scenarios**

PPD is water soluble, has a MW of 108.1, and has been shown to penetrate the skin by detection in plasma, urine and faeces after topical application of a PPD-containing hair dye.\(^ {31}\)

In a typical hair dye formulation, a precursor (e.g. PPD or PTD) and different couplers (e.g. resorcinol and \(m\)-aminophenol) are mixed with an oxidizing agent (hydrogen peroxide) under alkaline conditions, in order to form and attach the desired colour(s) within the hair shaft. Although there are only few studies available in the literature, they suggest a moderate penetration enhancement effect of a high pH.\(^ {32,33}\)

Hueber-Becker *et al.* showed in their study that the commercial \([^{14}\text{C}]\)PPD-containing oxidative hair dye which was applied to human volunteers for 30 min, had been excreted predominantly (90% of the total excreted PPD) via urine.\(^ {31}\) It should, however, be taken into account that excreted \([^{14}\text{C}]\)PPD, also includes \([^{14}\text{C}]\)PPD equivalents or derivatives. The majority of these derivatives are formed through \(N\)-acetylation of PPD to monoacetyl-PPD, which in turn is acetylated to diacetyl-PPD by the \(N\)-acyetyltransferase enzymes. This was shown *in vitro* by the use of cultured keratinocytes and cytosolic fractions of the human skin\(^ {34}\) and confirmed *in vivo* in humans by detection of these acetylation products in urine after the use of a PPD-containing hair dye.\(^ {31,35,36}\)

The detection of the acetylated PPD derivatives - which can only be formed intracellularly - in the urine of subjects who dyed their hair supports the uptake of PPD and the efficient release of acetylated PPD products.\(^ {35,36}\)

The results for \([^{14}\text{C}]\)PPD-containing hair dyes, as well as PPD dissolved in petrolatum (which is commonly used for patch testing), are summarized in Table 1. Table 2 provides a similar overview for the other compounds, discussed in relation to PPD penetration.
### Authors:
- Wolfram and Maibach (1985)
- Goetz et al. (1988)
- Hueber-Becker et al. (2004)

### Experimental design:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formulation</th>
<th>In vivo/ Ex vivo/ In vitro</th>
<th>Human/ Animal skin</th>
<th>Concentration (%)</th>
<th>Maximum dose applied (µg/cm²)</th>
<th>Exposure time (h)</th>
<th>Total recovery of applied dose (%)</th>
<th>Recovery of applied dose per compartment in µg_eq/cm² (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPD</td>
<td>[¹⁴C]PPD in oxidative hair dye formulation</td>
<td>In vivo</td>
<td>Human scalp</td>
<td>2.7</td>
<td>2700</td>
<td>0.33</td>
<td>0.19</td>
<td>ND</td>
</tr>
<tr>
<td>PPD</td>
<td>PPD in oxidative hair dye formulation</td>
<td>In vivo</td>
<td>Monkey scalp</td>
<td>2.7</td>
<td>2700</td>
<td>0.33</td>
<td>0.18</td>
<td>ND</td>
</tr>
<tr>
<td>PPD</td>
<td>[¹⁴C]PPD in oxidative hair dye formulation</td>
<td>In vivo</td>
<td>Human scalp</td>
<td>2.52</td>
<td>1449</td>
<td>NS*</td>
<td>0.04</td>
<td>ND</td>
</tr>
<tr>
<td>PPD</td>
<td>PPD in oxidative hair dye formulation</td>
<td>In vivo</td>
<td>Human scalp</td>
<td>2.0</td>
<td>1871</td>
<td>0.5</td>
<td>0.54</td>
<td>ND</td>
</tr>
<tr>
<td>PPD</td>
<td>PPD in oxidative hair dye formulation</td>
<td>Ex vivo</td>
<td>Human abdominal</td>
<td>1.99</td>
<td>398</td>
<td>0.5</td>
<td>3.71</td>
<td>ND</td>
</tr>
</tbody>
</table>

**Table 1** DPBS, Dulbecco’s phosphate buffered saline; MEL, measured exposure level (representing the amount of PPD equivalents found in stratum corneum, epidermis, dermis and receptor fluid); ND, not determined; NMM, new maintenance medium; NS, not specified; SC, stratum corneum; SLS, sodium lauryl sulphate.

For each study, the experimental conditions and the amounts of recovered PPD in the different compartments are listed. For convenience, only mean values (no standard deviations) are shown. Wherever recovery values were not expressed explicitly in the paper, they were derived from tables or figures, or calculated on the basis of values that were present.

*Not specified, but according to the manufacturer’s instructions. †Calculated on the basis of values given in the paper.
<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>PPD</td>
<td>PPD</td>
<td>PPD</td>
<td>PPD</td>
</tr>
<tr>
<td></td>
<td>[14C]PPD in ethanol/water (40/60 v/v)</td>
<td>[14C]PPD in DPBS and NMM media</td>
<td>[14C]PPD in petrolatum</td>
<td>[14C]PPD in oxidative hair dye formulation</td>
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<td>Ex vivo</td>
<td>In vivo</td>
<td>In vitro</td>
<td>Ex vivo</td>
<td>Ex vivo</td>
</tr>
<tr>
<td>Pig ear</td>
<td>Rat</td>
<td>Human reconstructed epidermis model</td>
<td>Pig</td>
<td>Pig</td>
</tr>
<tr>
<td>1.99</td>
<td>3.3</td>
<td>NS</td>
<td>1</td>
<td>2</td>
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<td>389</td>
<td>200</td>
<td>36.7</td>
<td>400</td>
<td>3000</td>
</tr>
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<td>0.5</td>
<td>4</td>
<td>0.5</td>
<td>48</td>
<td>0.5</td>
</tr>
<tr>
<td>5.1</td>
<td>♂ 0.71</td>
<td>12.5</td>
<td>51.3</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>♀ 3.7</td>
<td></td>
<td></td>
<td>4.59</td>
</tr>
<tr>
<td>7.31 (1.69)</td>
<td>ND</td>
<td>NS (&lt;0.2)</td>
<td>NS</td>
<td>NS (3.66)</td>
</tr>
<tr>
<td>13.19 (3.05)</td>
<td>ND</td>
<td>NS (&lt;0.2)</td>
<td>109.6 incl SC (27.4)</td>
<td>5.9 incl. SC (0.20) (0.63)</td>
</tr>
<tr>
<td>1.43 (0.33)</td>
<td>ND</td>
<td>4.59 (12.5)</td>
<td>95.5 (23.9)</td>
<td>0.9 (0.03) (0.3)</td>
</tr>
<tr>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>ND</td>
<td>♂ 1.41 (0.71)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>♀ 7.40 (3.7)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21.9 †</td>
<td>4.59 †</td>
<td>205.1</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>Open</td>
<td>No rinsing</td>
<td>DPBS</td>
<td>No rinsing</td>
<td>Water and shampoo</td>
</tr>
<tr>
<td>Water and SLS</td>
<td>OCCLUDED</td>
<td>Open</td>
<td>OCCLUDED</td>
<td>Open</td>
</tr>
<tr>
<td>24</td>
<td>4</td>
<td>24</td>
<td>72</td>
<td>72</td>
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<td></td>
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Table 1 (continued)
<table>
<thead>
<tr>
<th>Authors:</th>
<th>Kiese and Rauscher (1968)</th>
<th>Hruby (1977)</th>
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<tbody>
<tr>
<td>Experimental design:</td>
<td></td>
<td></td>
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<tr>
<td>Compound</td>
<td>PTD</td>
<td>PTD</td>
</tr>
<tr>
<td>Formulation</td>
<td>Oxidative hair dye formulation</td>
<td>[14C]PTD in oxidative hair dye formulation</td>
</tr>
<tr>
<td>In vivo/ ex vivo/ in vitro</td>
<td>In vivo</td>
<td>In vivo</td>
</tr>
<tr>
<td>Human/ Animal skin</td>
<td>human</td>
<td>dog</td>
</tr>
<tr>
<td>Concentration (%)</td>
<td>2.5</td>
<td>8.3</td>
</tr>
<tr>
<td>Maximum dose applied (µg/cm²)</td>
<td>3571</td>
<td>2000</td>
</tr>
<tr>
<td>Exposure time (h)</td>
<td>0.67</td>
<td>3</td>
</tr>
<tr>
<td>Total recovery of applied dose (%)</td>
<td>0.18</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Recovery of applied dose per compartment in µg eq/cm² (%)

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Str. Corneum</td>
<td>ND</td>
<td>ND</td>
<td>WBH:</td>
</tr>
<tr>
<td>- Epidermis + dermis</td>
<td>ND</td>
<td>ND</td>
<td>0.52 (0.063)</td>
</tr>
<tr>
<td>- Receptor fluid</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>- Urine</td>
<td>6.57 (0.18)</td>
<td>1.84 (0.092)</td>
<td>1.17 (0.14)</td>
</tr>
<tr>
<td>- Faeces</td>
<td>ND</td>
<td>16.8 (0.84)</td>
<td>0.03 (0.004)</td>
</tr>
</tbody>
</table>

Maximum recovery in plasma (Cmax in µg eq/ml) of the applied dose per cm² (%)

<p>| | | | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Application</td>
<td>Open</td>
<td>Open</td>
<td>Open</td>
</tr>
<tr>
<td>Rinsing</td>
<td>Water</td>
<td>Water</td>
<td>Water and shampoo</td>
</tr>
<tr>
<td>Collection time post-application (h)</td>
<td>48</td>
<td>96</td>
<td>24</td>
</tr>
</tbody>
</table>

Table 2 DPBS, Dulbecco’s phosphate buffered saline; ND, not determined; NMM, new maintenance medium; NS, not specified; SCCP, Scientific Committee on Consumer Products; SLS, sodium lauryl sulphate; WBH, whole body homogenate. For each study, the experimental conditions and the amounts of recovered PTD or PAP in the different compartments are listed. For convenience, only mean values (no standard deviations) are shown. Wherever recovery values were not expressed explicitly in the paper, they were derived from tables or figures or calculated on the basis of values that were present.

<table>
<thead>
<tr>
<th>PAP</th>
<th>PAP</th>
<th>PTD</th>
</tr>
</thead>
<tbody>
<tr>
<td>[^{14}\text{C}]PAP in DPBS and NMM media</td>
<td>[^{14}\text{C}]PTD in hair dye formulation without developer</td>
<td>[^{14}\text{C}]PTD in oxidative hair dye formulation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>In vivo</th>
<th>In vitro</th>
<th>Ex vivo</th>
<th>Ex vivo</th>
<th>Ex vivo</th>
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<tbody>
<tr>
<td>rat</td>
<td>human reconstructed epidermis model</td>
<td>pig</td>
<td>pig</td>
<td>human</td>
</tr>
<tr>
<td>1.5</td>
<td>NS</td>
<td>4.6</td>
<td>5.4</td>
<td>4.5</td>
</tr>
<tr>
<td>75</td>
<td>1.5</td>
<td>4600</td>
<td>1080</td>
<td>900</td>
</tr>
<tr>
<td>24</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>0.67</td>
<td>9</td>
<td>0.23</td>
<td>2.39</td>
<td>3.4</td>
</tr>
</tbody>
</table>

| ND          | ND               | ND      | ND      | ND      |
| ND          | ND               | Upper dermis 4.9 | 13.5 (1.25) | Including receptor |
| ND          | 0.14 (9)         | (0.11) | 14.9 (1.38) | fluid 31.3 (3.4) |
| ND          | ND               | 5.6 (0.12) | ND      | ND      |
| ND          | ND               | ND      | ND      | ND      |
| ND          | ND               | ND      | ND      | ND      |

Table 2 (continued)

**In vivo studies**

_Human urinary excretion._ An early report by Wolfram and Maibach in 1985 showed that application of a commercial oxidative hair dye formulation containing 2.7% \[^{14}\text{C}]PPD to human scalp hair for 20 min resulted in a total urinary excretion of 0.19% ± 0.06 % of the applied dose (Table 1), with a half-time of 16 h.\(^{37}\) These data were confirmed by Goetz _et al._, examining percutaneous absorption after hair dying (n = 5 females) with an unlabelled PPD-containing hair dye formulation.\(^{35}\) Approximately 85% of the amount recovered in 48 h was released during the first 24 h. Their analytical method allowed the determination of PPD derivatives in urine, and the authors calculated that 80% of their detected PPD derivatives actually consisted of diacetyl-PPD. Taking into account the different doses used, the total dose
excretion (0.04% after 24 h) was similar to that of Wolfram and Maibach (Table 1).\textsuperscript{37} Regarding kinetics, they observed acetylated PPD derivatives in urine by 30 min after the typical dyeing procedure had been completed. Hueber-Becker et al. used a detailed mass balance approach, with standard operating procedures and defined conditions, such as hair length, professional hairdressers and the collection of clipped-off hairs, washing water, and used materials, to investigate human systemic exposure after application of a specifically prepared 2\% [\textsuperscript{14}C]PPD-containing oxidative hair dye to the scalp.\textsuperscript{31} This was determined by measuring the mean radioactivity excreted in blood, urine and faeces in different intervals, for up to 120 h after application. The total excretion was 0.54\% ± 0.25\% of the applied dose (Table 1). Again, the majority (86\%) of absorbed radioactivity was excreted within 24 h after application. By using the same human subjects and experimental conditions, Nohynek et al. revealed that the majority, namely 80-95\% of the PPD metabolites excreted via the urine consisted of monoacetyl-PPD and diacetyl-PPD, confirming the previous results of Goetz et al.\textsuperscript{36} Two recent human \textit{in vivo} studies - not shown in Table 1 because details were not available - summarized by the Scientific Committee on Consumer Safety (SCCS) obtained excretion data similar to those of the previous studies. Briefly, both studies applied a [\textsuperscript{14}C]PPD containing hair dye (1\% PPD and 2\% PPD, respectively) for 30 min to human volunteers under standardized conditions, with the application being performed by professional hairdressers. Urinary excretion was 0.88\% ± 0.46\% of the applied dose in the 1\% PPD study and 0.72\% ± 0.25\% of the applied dose in the 2\% PPD study. The study using 1\% PPD also investigated the composition of urinary PPD equivalents. This showed that >99\% consisted of diacetyl-PPD, and that some urine samples contained very low levels of PPD (<0.3\%) or monoacetyl-PPD (<0.2\%). Furthermore, some of the urine samples showed traces of the completed hair dye product (trimer) as well as the monoacetylated trimer.\textsuperscript{45} The values in the above-mentioned studies are well in line with values found for PTD, another hydrophilic aromatic amine that is also used as a precursor in oxidative hair dyes (Table 2). Topical application of a PTD-containing (home-made) hair dye to human volunteers for 40 min resulted in a urinary excretion of 0.18\% of the applied amount (estimated at 3571 µg/cm\textsuperscript{2}).\textsuperscript{42} The urinary excretion peaked in the 4 h post-application, and 90\% of all the excreted PTD equivalents over 48 h were detected within 24 h.

\textit{Human plasma levels.} Hueber-Becker et al. additionally examined plasma concentrations and showed that the maximal PPD levels (C\textsubscript{\text{max}}) of 0.087 µg\textsubscript{eq}/ml (i.e. 0.0046\% of the applied dose per cm\textsuperscript{2}) were found at approximately 2 h (T\textsubscript{\text{max}}) after dyeing, whereas after 24 h no more [\textsuperscript{14}C]PPD equivalents could be detected.\textsuperscript{31} This is in accordance with the urinary excretion of PPD, which has also been shown to be most frequently completed within 24 h. The two studies summarized by the SCCS,\textsuperscript{45} also examined PPD equivalent plasma kinetics following the hair dye application. It was shown, for both concentrations (1\% PPD and 2\% PPD) that the
respective $C_{\text{max}}$ of 0.0974 and 0.133 $\mu g_{eq}/ml$ were found at $T_{\text{max}}$ of 2 h, which is in concor-
dance with the study performed by Hueber-Becker et al. Furthermore, in the hair dye pro-
dure with 1% PPD it was shown that the PPD equivalents in plasma consisted of diacetyl-PPD,
and no PPD or monoacetyl-PPD was detected. A few plasma samples showed traces of the
completed hair dye products (or their acetylated part) at amounts slightly above their lower
limits of quantification.

Animal urinary excretion. In addition to the human scalp, Wolfram and Maibach applied the
2% $[^{14}\text{C}]$PPD-containing oxidative hair dye to the scalp of rhesus monkeys. The total urinary
excretion of 0.18% ± 0.06% was similar to the excretion values for humans (0.19% ± 0.06%;
collection time both species up to 144 h; Table 1). Again, these values were similar to those
following PTD application, with 0.09% of the applied dose (2000 $\mu g/cm^2$) and 0.14% of the
applied dose (833 $\mu g/cm^2$) being excreted in the urine by dogs and rats, respectively
(Table 2).

Animal plasma levels. Dressler and Appelqvist investigated the plasma pharmacokinetics of
dermal PPD exposure in rats. Three male and three female rats, clipped free of hair, were
exposed under occlusion to 200 $\mu g/cm^2$ $[^{14}\text{C}]$PPD solution for 4 h. Analysis of plasma taken
after 4 h showed the exclusively presence of diacetyl-PPD. In males the mean plasma level was
1.41 ± 0.34 $\mu g_{eq}/ml$ (0.71% of applied amount per cm$^2$), whereas in females a higher mean
plasma level of 7.40 ± 1.83 $\mu g_{eq}/ml$ was reached (3.7% of applied amount per cm$^2$), which
was not attributable to a true sex difference, but to the difference in body weight (Table 1). For $p$-
aminophenol, another hydrophilic aromatic amine used in hair dyes and investigated in
this study, the mean maximal plasma level at $T_{\text{max}}$ 4 h of 0.50 $\mu g_{eq}/ml$, corresponding to 0.67%
of the applied amount per cm$^2$, was similar to the level of PPD, despite different experimental
conditions (Table 2).

Summarising these in vivo data, it can be stated that, under simulated hair dye conditions,
0.04% - 0.88% of the applied PPD-containing dye formulation is excreted. Whereas the
Hueber-Becker et al. study was the only one to investigate both urinary and faecal excretion,
they showed that absorbed PPD is excreted primarily (>90%) via urine. Furthermore, these
data show that most of the absorbed PPD had been excreted within 24 h. However, a study
by White et al. showed persistence of PPD equivalents in the stratum corneum for as long as
3 days after an only very short application (5 min) of a PPD-containing hair dye. The PPD
was probably bound to the keratins, as these are the major proteins in the stratum corneum.
This part may then act as a reservoir, although limited in duration, as the turnover time of the
stratum corneum is approximately two weeks. However, the relevance of a PPD reservoir in
the stratum corneum for sensitization remains unknown, as the amount of PPD bound to this
layer under hair dye conditions and the release capacity have not been established. Penetration through the skin, and hence the appearance in plasma and urine, was monitored shortly after the hair dyeing procedure, and PPD metabolites were found in urine by 30 min after completion of the dyeing procedure. Moreover, the majority of excreted PPD was found to have been acetylated to monoacetyl-PPD and diacetyl-PPD. Three studies examined human \textit{in vivo} plasma concentrations, and all showed that PPD equivalents reached maximal plasma concentrations 2 h after application of the hair dye. Animal plasma levels were somewhat higher compared to human plasma values, probably reflecting the longer application time, the different formulation, or the higher level of penetration through rat skin than through human skin.\textsuperscript{30} The relatively low amount of PPD that had penetrated, and hence considered to be bioavailable considered PPD (0.04 – 0.88% of applied dose), in the \textit{in vivo} experiments resembling conditions of permanent hair dyeing, are well in line with the reviewed data on percutaneous absorption of PTD and \textit{p}-aminophenol, as well as with data for many other oxidative hair dye amines compiled by Platzek.\textsuperscript{48}

\textbf{In vitro and \textit{ex vivo} studies}

\textit{In vitro study: human reconstructed epidermis model}. In general, penetration is considered to be higher in these models than \textit{in vivo} experiments. This points to incomplete barrier function in these models.\textsuperscript{49,50} Hu \textit{et al.} investigated the transformation of various amounts of PPD and \textit{p}-aminophenol in the EpiDerm\textsuperscript{TM} model, consisting of human reconstructed epidermis.\textsuperscript{39} Topical application was studied in the case of \textit{p}-aminophenol, whereas both topical application and exposure via culture media were examined in the case of PPD. Both PPD and \textit{p}-aminophenol exposure resulted in dose dependent \textit{N}-acetylation. \textit{p}-Aminophenol (total recovery in medium of 9%; Table 2) was completely acetylated within 24 h following topical application of 87 µl of 100 µM \textit{p}-aminophenol (equivalent to 1.5 µg/cm\(^2\)). Recovery of PPD and its acetylated metabolites in media following 24 h exposure via cell culture media showed approximately 80% acetylated compounds with 20 µM PPD, while the presence of 1000 µM resulted donor-dependently in 75-86% recovery of the unmodified parent compound. In the middle range, with 24 h exposure to 1 ml of 100 µM PPD (equivalent to 11 µg/ml PPD), approximately 50-70% was metabolised to acetylated products. For 30 min topical applications a similar concentration-dependent decline in acetylation was seen. Whereas topical exposure to 100 µM PPD resulted in complete acetylation with no parent PPD left, application of the highest PPD amount tested (2500 µM, corresponding to a dose of 36.7 µg/cm\(^2\)) with subsequent rinsing, followed by a 23.5 h recovery period of the model in culture medium, resulted in 7.1% acetylation (recovery: 1.2% as monoacetyl-PPD, 5.9% as diacetyl-PPD and 5.3% recovery of unmetabolised PPD within 24 h) (12.5% recovery in total; Table 1). The observed reduction of acetylation in the presence of increasing PPD concentrations points to enzyme saturation and inhibition of \textit{N}-acetylation capacities.\textsuperscript{39} This modulation was also shown
in an immortalized keratinocyte cell line (HaCat) and in normal human epidermal keratinocytes by Bonifas et al.\textsuperscript{51} It is very likely that, also in case of application of temporary black henna tattoos, saturation of the N-acetylation pathway occurs, as a high concentration of PPD is often added and PPD is present on the skin for a long period.\textsuperscript{52,53}

**Human and animal ex vivo studies.** Goebel et al. compared exposure levels of [$^{14}$C]PPD under different conditions, using pig skin samples in flow-through diffusion cells.\textsuperscript{40} A 48 h occlusive PPD patch test (dose of 400 µg PPD/cm\(^2\)) was compared with a simulated 30 min open hair dyeing procedure (dose of 3000 µg PPD/cm\(^2\)). The total absorption, measured in the stratum corneum, epidermis, dermis and receptor fluid, was 205.1 µg\textsubscript{eq}/cm\(^2\) (51.3 % of the applied dose) for the patch test, and 6.8 µg\textsubscript{eq}/cm\(^2\) (0.23 % of the applied dose) for the hair dye formulation (Table 1). A lower dose (± 400 µg/cm\(^2\)) with a very similar hair dyeing approach and with the use of both human abdominal and pig ear skin, was tested by Hueber-Becker et al. (Table 1).\textsuperscript{31} Twenty-four hours after a 30 min open application procedure, the amount of PPD that had penetrated was determined in the skin and the receptor fluid. The total amount per surface area absorbed could then be calculated at 16.1 µg\textsubscript{eq}/cm\(^2\) (3.71 % of applied dose) for human skin and at 21.9 µg\textsubscript{eq}/cm\(^2\) (5.1 % of applied dose) for pig skin, which is approximately 10-fold higher than the values in their in vivo study (total absorption of 0.54 % of applied dose) and also in line with the hair dye formulation of Goebel et al. (0.23 % of the applied dose).\textsuperscript{40} Another human ex vivo study, summarized by the SCCS (not included in Table 1), examined percutaneous absorption of [$^{14}$C]PPD in the presence or absence of other dyes, of a developer and/or of human hair, using PPD doses ranging from 540-2700 µg/cm\(^2\). Remarkably, the penetration in all conditions was between 0.1 and 0.2 % of the applied dose and the presence of hair on the surface did not significantly influence penetration.\textsuperscript{45} In a study of Steiling et al. 30 min of application of a [$^{14}$C]PPD containing hair dye formulation resulted in recovery of 4.59 % of the applied dose in the skin and receptor fluid (Table 1).\textsuperscript{41} In conclusion, the experiments modelling hair dye conditions (similar exposure time of 0.5 h and using an oxidative hair dye formulation) show total absorptions ranging from 0.23 % - 5.1 % of the applied dose when using ex vivo pig skin was used and 0.1 - 3.71 % when using ex vivo human skin was used. These values are in concordance with calculated absorption rates ranging from 0.04 % to 0.88 % of the applied dose when in vivo human skin was used. The use of a different condition - namely a patch test condition that involves occlusion, a longer exposure time of 48 h, and the vehicle petrolatum - shows a considerable impact on the absorption, which could be calculated at 51.3 % of the applied dose. For PTD percutaneously applied to pig skin, absorption values were similar to PPD values, and ranged from approximately 0.23 % to 2.39 % of the applied dose, whereas 3.4 % of the applied dose penetrated ex vivo human skin (Table 2).\textsuperscript{44} The ‘measured exposure level’ (MEL) provides a better estimate of the actual exposure than calculating the amount of the applied dose that has penetrated. The MEL, consisting of the
amount of chemical in the stratum corneum, epidermis, dermis and receptor fluid, was introduced by Goebel et al., but could also be calculated for three other studies on the basis of the data provided (Table 1). These MELs again show the major impact of exposure time, as the higher MEL for the patch test application was obtained with a dose similar to the dose used in the studies of Hueber-Becker et al., which resulted in ten-fold lower MELs.

Conclusions

Despite the different conditions used (i.e. formulation, species, and applied doses) in the reviewed PPD studies, the penetration values upon a short exposure time of approximately 0.5 h are quite similar. When considering daily life exposure to PPD (e.g. hair dyeing), one may consider that 0.04% - 0.88% of the applied dose penetrates the skin. Furthermore, the reviewed studies indicate that PPD is metabolized to monoacetyl-PPD and diacetyl-PPD, but N-acetylation capacities decrease with increasing dose of PPD. Although only small amounts of PPD penetrate the skin during a 0.5 h exposure, this is enough to induce sensitization in susceptible individuals, as well as to elicit allergic contact dermatitis in previously sensitized subjects. This may relate to potency and/or to the short timescale of immunological events after topical application. Although penetration remains a prerequisite, the chemical and protein reactivity of a chemical have been proposed to be major determinants for the induction of contact sensitization. Therefore, they form the subject of the following part of this review.

Chemical reactivity and protein binding

To date, the murine local lymph node assay (LLNA) has been considered the preferred skin sensitization prediction model. However, the clear need for non-animal based methods has resulted in the development of a wide variety of in vitro, in silico and in chemico approaches for risk assessment. All of these methods refer to one of the key steps in the sensitization cascade; allergen penetration, binding to proteins (i.e. haptenation), stimulation of antigen presenting cells, or T cell proliferation. Nevertheless, recent studies have shown that reactivity of the hapten with peptides or proteins is a major determinant in predicting allergenic potency. Actually, the protein reactivity of the allergens investigated thus far has been shown to correspond very well with the allergen’s sensitizing potency as determined by the LLNA’s EC3 value (the concentration giving a stimulation index of 3). This correlation between protein reactivity and sensitization has been known for many years. In 1935, Landsteiner and Jacobs described the classical ‘hapten concept’, which stated that a chemical needs to bind covalently to skin proteins to form hapten-protein complexes, in order to become immunogenic and to start the sensitization cascade. In addition to this initial hapten
concept regarding skin sensitization, the pro-hapten and pre-hapten concepts arose. The pro-hapten concept states that chemicals that are initially not chemically reactive towards proteins have to be metabolized before being able to haptenate protein and to become immunogenic. Nevertheless, as discussed by Lepoittevin, not all non-protein-reactive chemicals are transformed enzymatically; some are, for example, non-enzymatically oxidized. He therefore suggested a modification of the concept and introduced the pre-hapten concept, for ‘non-reacting sensitizing molecules transformed into haptens by simple chemical transformation and without requirement of a specific enzymatic system’. Considering that, depending on the environmental conditions, pre-haptens may behave as pro-haptens, and that there are no validated methods to distinguish a pre-hapten from a pro-hapten in vivo or ex vivo, the use of these distinctive concepts has been challenged. Nevertheless, they are still in common use to describe the different pathways needed for a chemical to haptenate proteins. Even though several complex chemical mechanisms may underlie the haptenation process, it is thought to mainly occur through an electrophilic-nucleophilic interaction. The electrophilic moieties on haptens enable them to covalently interact with nucleophilic side chains of amino acids of cutaneous protein. In recent years, several chemical reactivity assays have been developed. Most of them measure the depletion of the protein or peptide, that is, the nucleophile. However, additional analysis of the formed adducts may reveal supplementary information on reactivity, and can exclude false positive peptide depletion results based on chemical-induced peptide dimerization. The experimental nucleophilic compound often consists of a mixture of amino acids, is a synthetic model peptide, or is a naturally present protein, such as human serum albumin (HSA) or glutathione (GSH). There are several analytical methods available to detect adduct formation with exact localization of protein modification, as well as to determine nucleophile depletion; these have been elegantly summarized by Gerberick et al. In addition to the existing peptide-based reactivity assays, Gerberick et al. introduced an assay integrating enzymatic activity, allowing the detection of pro-haptens. This is an important addition, considering the fact that at least one-third of the known sensitizers are believed to be not directly protein-reactive. Furthermore, it is probable that not all hapten-protein adducts formed are immunogenic, but, to date, studies investigating this are lacking. Although the side chains of many amino acids in protein contain nucleophiles that are capable of reacting with electrophilic allergens, in vitro data suggest that chemicals show selectivity in their amino acid preference. In fact, although histidine, methionine and tyrosine can react with electrophiles, reactivity assays using cysteine and lysine show the highest depletion and a stronger correlation with LLNA EC3 values.

Haptenation: what is known about PPD
PPD is regarded as not directly protein-reactive (i.e. non-electrophilic), and may be classified as pre-hapten. In general, there is not much certainty regarding either the nature
of the hapten-protein complex or the way in which it stimulates immune cells. For PPD, it is even harder to predict the interaction with protein, owing to its intrinsic instability and rapid auto-oxidation when it is exposed to air oxygen or is present in aqueous solution. Its primary oxidation product, \( p \)-benzoquinonediimine, is susceptible to sequential oxido-conjugation reactions, ultimately leading to formation of the trimer Bandrowski’s base (Figure 1). In addition to PPD, \( p \)-benzoquinonediimine and Bandrowski’s base, Aeby et al. found another dimeric structure and two trimeric structures. This was, however, achieved after \textit{in vitro} experiments with exaggerated oxygenation. Furthermore, \( p \)-benzoquinonediimine is susceptible to hydrolysis, generating \( p \)-benzoquinones (Figure 1). As well as this non-enzymatic formation of reactive oxidation products, PPD has been shown to be enzymatically converted into the acetylation products monoacetyl-PPD and diacetyl-PPD by \textit{N}-acetyltransferase 1 (NAT1), a phase II enzyme abundant in epidermal keratinocytes (Figure 1). In contrast to the oxidized derivatives of PPD, the acetylated products monoacetyl-PPD and diacetyl-PPD are negative in the LLNA (standard LLNA according to Organization for Economic Co-operation and Development, guideline 429). Furthermore, they have been shown to be incapable (monoacetyl-PPD) or less capable (diacetyl-PPD) of eliciting allergic reactions when PPD-sensitized subjects are patch tested. However, the acetylation capacities of keratinocytes may become saturated or inhibited under certain circumstances. Hence, disturbing the precarious balance between acetylation and oxidation might be critical in inducing sensitization to PPD. Furthermore, because of PPD’s instability and the excellent auto-oxidation conditions provided by the air-exposed skin surface, it is very possible that PPD already oxidizes before it enters the skin and before it has the chance to become acetylated (Figure 1). Actually, it has been shown \textit{in vitro} that, 30 min after exposure to oxygen in air, another monomer, possibly \( p \)-benzoquinone-diimine, can be detected. This may thus expose the skin to the highly protein-reactive oxidatized intermediates, although \( p \)-benzoquinonediimine might be considered to be too reactive to penetrate the stratum corneum. For Bandrowski’s base, the penetration may also be limited, at least for human patch test conditions with single-dose exposure, because although Bandrowski’s base is an extreme sensitizer in the LLNA (approximately 10-fold more sensitizing than PPD), and is capable of activating dendritic and THP-1 cells \textit{in vitro} and stimulating T cell proliferation of PPD-allergic patients \textit{ex vivo}, only 16% of PPD patch test-positive patients responded weakly to Bandrowski’s base when the latter was applied under patch test conditions. Furthermore, hair dye conditions - providing an alkaline environment with an excess of couplers and only a limited exposure time - prompt the formation of less permeable colouring molecules rather than the PPD auto-oxidation products \( p \)-benzoquinonediimine and Bandrowski’s base (Figure 1). To summarize, it is suggested that when NAT1 acetylation capacity is not sufficient, a range of subsequently formed PPD oxidation products may result in the generation of a spectrum of known and unknown electrophilic and hence antigenic determinants (Figure 1). As well as the electrophilic
\( p \)-benzoquinonediamine, other oxidized derivatives, such as \( p \)-benzoquinone, Bandrowski's base, and PPD itself, have been subjected to protein reactivity assays. Nevertheless, when studying PPD in these assays, one has to bear in mind that PPD itself is not protein-reactive, and that, actually, one of the auto-oxidation derivatives, probably the primary oxidation product \( p \)-benzoquinonediamine, reacted to the investigated protein. 

**Figure 1** Overview of the proposed penetration and conversion of \( para \)-phenylenediamine (PPD) on and in the skin(model). PPD is known to penetrate the skin and here it is subjected to \( N \)-acetylation or oxidation. Oxidation of PPD may lead to formation of a \( para \)-benzoquinonediamine intermediate (p-BQDI), which may be transformed into \( para \)-benzoquinone (p-BQ) through hydrolysis or into Bandrowski's Base (BB) through several oxido-conjugation reactions. These PPD-oxidation derivatives have been shown to react to amino acid residues of proteins (in \textit{in vitro} experiments) and might be the complexes responsible for sensitization. \( N \)-acetylation on the other hand, will form monoacetyl-PPD (MAAPPD) and diacetyl-PPD (DAAPPD), which are considered non-sensitizing compounds (in the LLNA). Skin conditions may allow formation of the oxidative derivatives on the skin surface, although their penetration into the skin might be limited because of their reactivity. Thick arrow: significant penetration, dotted arrows: proposed steps, not confirmed \( in \textit{vivo} \).
**PPD intermediates reacting with amino acids**

Eilstein *et al.* were able to show that 2,5-$[^{13}C]$-dimethyl substituted $p$-benzoquinonediimine reacted with several nucleophilic amino acids (e.g. *N*-acetyl-cysteine, *N*-acetyl-tryptophan and *N*-acetyl-lysine) through a set of complex chemical mechanisms. The two $^{13}C$-labelled methyl groups were introduced on the aromatic ring of $p$-benzoquinonediimine, because this facilitates the study of its protein reactivity by specific mass spectrometric techniques. Whereas cysteine was directly and covalently bound to 2,5-$[^{13}C]$-dimethyl-$p$-benzoquinonediimine, tryptophan and lysine were first subjected to oxidation and reduction processes. The authors concluded that the formed adducts deserved serious consideration as potential antigenic structures responsible for contact sensitization. In 2007, this group published the results of a follow-up study, wherein the specific reactivity of 2,5-$[^{13}C]$-dimethyl-$p$-benzoquinonediimine towards lysine was investigated. The authors studied lysine in particular, because of its proposed key role in contact sensitization, together with the fact that cysteine reactivity does not necessarily relate to sensitizing potential, and the results of an *in vitro* study showing that, intracellularly, thiol groups of cysteine were less abundant than amino groups of lysine. They showed once more that, besides the known classical electrophilic-nucleophilic mechanism, complex oxidoreduction processes were observed. Actually, the 2,5-$[^{13}C]$-dimethyl-$p$-benzoquinonediimine oxidatively removed the amine group from lysine, revealing the reactive intermediate that is capable of adduct formation. Consequently, the authors concluded that, in addition to the direct covalent hapten-protein coupling, oxidative modification of proteins induced by $p$-benzoquinonediimine is an important determinant in the formation of antigenic structures, and hence could be relevant in sensitization to PPD. Likewise, Aptula *et al.* described two parallel chemical reaction mechanisms, thereby particularly emphasizing the importance of the free radical binding mechanism in the skin sensitization properties of PPD. They designated the sulfhydryl (SH) group (of cysteine) as the major reactor in hapten-protein binding. The reactivity towards amino acids has also been examined for other PPD intermediates. Peptide depletion assays showed that $p$-benzoquinone depleted lysine, cysteine, and histidine, whereas the trimer BB was shown to deplete cysteine and, to a lesser extent, lysine. PPD was shown to react with lysine, cysteine, histidine, and arginine. However, as discussed and shown by adduct analysis, the compound responsible for this reactivity should actually be assumed to be $p$-benzoquinonediimine or another oxidized derivative of PPD.

**PPD intermediates reacting with peptides**

By studying the exploratory compound 2,5-$[^{13}C]$-dimethyl-$p$-benzoquinonediimine with a cysteine-free model peptide (resembling the *N*-terminal chain of the globin protein), Eilstein *et al.* confirmed their previous findings on reactivity of this compound with lysine. Cysteine was excluded because of the known high reactivity of the thiol (SH) groups with allergens; exclusion of cysteine would potentially allow other potential amino acids to react. Furthermore,
in this way, the easily occurring cysteine dimerization was also avoided. Jenkinson et al. showed that in a more complex chemical environment (using model peptide DS3), p-benzoquinonedimine, as well as 2,5-dimethyl-p-benzoquinonedimine, were found to bind exclusively to cysteine, via a classical nucleophilic-electrophilic reaction. These experiments addressed the major effects of the environment on the binding capacity of a chemical. This capacity is highly influenced by the pH of the microenvironment, which, in turn, is dependent on the presence of surrounding amino acid. Currently, it is unknown whether hair dyeing can affect the pH in the skin layers, such that it is relevant for haptenation. In addition to p-benzoquinonedimine, the derivative p-benzoquinone was studied, and was shown to cause depletion of GSH, which was used as a cysteine-containing peptide.

PPD intermediates reacting with model proteins

In order to further characterize the specific amino acid modification of p-benzoquinonedimine, Jenkinson et al. used the His-tagged glutathione S-transferase π (His-GSTP) protein, and showed that both p-benzoquinonedimine and 2,5-dimethyl-p-benzoquinonedimine selectively and exclusively modified cysteine 47 (Cys47). No other cysteines (i.e. Cys14, Cys17, Cys101, and Cys169) were modified, reflecting their difference in reactivity caused by the specific physicochemical parameters. This exclusive binding to cysteine is in line with the results obtained with the model peptide DS3. Besides His-GSTP, Jenkinson et al. investigated the possible binding sites of PPD on HSA. They showed that PPD irreversibly bound to HSA at Cys34, but not to lysine and tryptophan, which also indicates that this covalent modification of cysteine is favoured over the other possible pathways. A mass spectrometric analysis suggested that a single molecule of the primary PPD oxidation product, p-benzoquinonedimine, had been added to HSA.

Conclusions

The modification of cutaneous proteins by a hapten is considered to be one of the prerequisites for the induction of contact sensitization, and this correlation has been acknowledged for many years. Therefore, a variety of protein reactivity assays have been designed, often using peptide depletion or adduct formation as outcome parameters. Specific mass spectrometric techniques have shown preferred amino acids, although this has been shown to be largely dependent on the micro-environment. Therefore, it should be stressed that these assays only predict in vitro reactivity and that the in vivo relevance of these adducts cannot be determined straightforwardly. In fact, this might be true for p-benzoquinone and Bandrowski’s base. Although both substances are positive in the LLNA and have been shown to form adducts with proteins in vitro, clinical studies suggest that they are not important in allergic contact dermatitis to PPD. Several studies showed the distinctive, complex mechanisms responsible for adduct formation. Whereas direct covalent binding through electrophilic-nucleophilic interactions was often
observed, complex oxidoreduction reactions were also shown to be involved. In the case of PPD, the (known) protein-reactive oxidized intermediates consist of \( p \)-benzoquinonediimine, \( p \)-benzoquinones and Bandrowski's base, which were most often found to react with the cysteine and lysine, although histidine, arginine and tryptophan might also play a role in the haptenation process. Some authors found that PPD or \( p \)-benzoquinones exclusively bound to specific cysteines in experiments using model peptides and proteins, and therefore suggested that cysteine may be an essential amino acid in contact sensitization to PPD.\(^{69,75,94}\) As discussed by Divkovic \textit{et al.}, prediction of the immunogenicity of particular protein modifications and, especially, determination of the exact peptide fragments that are presented to the naïve T cells would be extremely helpful in predicting the hapten's \textit{in vivo} allergenic effect.\(^77\) Moreover, it has been widely acknowledged that a combination of data from \textit{in silico}, \textit{in vitro} and \textit{in chemico} assays will be required to predict skin sensitization potential as accurately as possible.\(^{58,92}\) The first attempt of such a combined approach has been made.\(^92\)

**Concluding remarks**

As shown by the papers that have been reviewed, PPD remains an intriguing allergen because of its unstable character, which allows the formation of several protein-reactive auto-oxidation products. On the other hand, the human body seems to be very well protected against the formation of these potentially harmful oxidation products, by converting PPD into the detoxification products monoacetyl-PPD and diacetyl-PPD. The penetration of PPD has been shown to be dependent on the conditions used, especially the exposure-time. The small amount of PPD that penetrates after application to the skin (with the use of hair dye formulations and conditions), together with the fact that the majority of it becomes acetylated, shows the strong sensitizing potency of the remaining oxidized PPD derivatives. This is reflected in the prevalence figures. Whereas, in unselected dermatitis patients, the prevalence of PPD sensitization ranged from 1% to 6%, the prevalence increased to 38-97% in a highly selected hair dye dermatitis population, as shown by a literature review concerning European, North American and Asian data.\(^93\) Although, for the \textit{in vivo} situation, it is still not completely determined which amino acids PPD preferentially binds to, the results of several studies discussed in this review give important clues. However, there may be many unknown (oxidative) PPD derivatives, the impact of which is unpredictable. Thus, further experiments to define the specific antigenic adducts responsible for naïve T cell activation are needed to improve our understanding of the mechanism of PPD sensitization.
References


46. White JM, Basketter DA, Pease CK et al. Intermittent exposure to low-concentration paraphenylenediamine can be equivalent to single, higher-dose exposure. Contact Dermatitis 2007; 56: 262-5.


48. Platzek T. Risk from exposure to arylamines from consumer products and hair dyes. Front Biosci (Elite Ed) 2010; 2: 1169-83.


64. Dupuis G, Benezra C. Allergic contact dermatitis to simple chemicals. A Molecular Approach , New York, Marcel Dekker, 1982
65. Lepoittevin JP. Metabolism versus chemical transformation or pro- versus prehaptens? Contact Dermatitis 2006; 54: 73-4.
67. Aptula AO, Roberts DW, Pease CK. Haptens, prohapten and prehapten, or electrophiles and proelectrophiles. Contact Dermatitis 2007; 56: 54-6.


