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Title: Skin sensitization quantitative risk assessment for occupational exposure of hairdressers to hair dye ingredients

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Abstract:

Occupational exposure of hairdressers to hair dyes has been associated with the development of allergic contact dermatitis (ACD) involving the hands. *p*-Phenylenediamine (PPD) and toluene-2,5-diamine (PTD) have been implicated as important occupational contact allergens. To conduct a quantitative risk assessment for the induction of contact sensitization to hair dyes in hairdressers, available data from hand rinsing studies following typical occupational exposure conditions to PPD, PTD and resorcinol were assessed. By accounting for wet work, uneven exposure and inter-individual variability for professionals, daily hand exposure concentrations were derived. Secondly, daily hand exposure was compared with the sensitization induction potency of the individual hair dye defined as the No Expected Sensitization Induction Levels (NESIL). For PPD and PTD hairdresser hand exposure levels were 2.7 and 5.9 fold below the individual NESIL. In contrast, hand exposure to resorcinol was 50 fold below the NESIL. Correspondingly, the risk assessment for PPD and PTD indicates that contact sensitization may occur, when skin protection and skin care are not rigorously applied. We conclude that awareness of health risks associated with occupational exposure to hair dyes, and of the importance of adequate protective measures, should be emphasized more fully during hairdresser education and training.

Key words:

hair dye allergy, allergic contact dermatitis, skin sensitization, quantitative risk assessment, hairdresser, occupational exposure, *p*-phenylenediamine, resorcinol, toluene-2,5-diamine

Abbreviations:

ACD, allergic contact dermatitis; BAuA, German federal institute for occupational safety and health; DK-EPA, Danish environmental protection agency; EC3, effective concentration of the test substance required to produce a three-fold increase in the stimulation index compared to vehicle-treated control; ECHA, European chemical agency; ECETOC, European centre for

49 ecotoxicology and toxicology of chemicals; EU, European Union; EU-OSHA, European agency
50 for safety and health at work; HSE, Health and safety executive of the United Kingdom; IKW,
51 German cosmetic, toiletry, perfumery and detergent association; IVDK, Information Network of
52 Departments of Dermatology in Germany, Switzerland, and Austria; LLNA, local lymph node
53 assay; MEL, measured exposure level; NESIL, no expected sensitization induction level; OECD,
54 organization for economic co-operation and development; PPD, para-phenylenediamine; PTD,
55 toluene-2,5-diamine; QRA, quantitative risk assessment; SAF, sensitization assessment factor;
56 SC, stratum corneum; SCCS, scientific committee for consumer safety; WHO, world health
57 organization; WoE, weight-of-evidence

58

Introduction

The hairdressing sector workforce in Europe is greater than one million people who are employed in approximately 400,000 salons. Hairdressing is predominantly a female profession (more than 80 % of the workforce are female), and is also a predominantly young workforce with about 80 % of the recruits being under the age of 26, and about 56 % under 19.

Risk factors for skin sensitization of the hairdresser profession have been well documented. These include frequent contact with water (wet work) which - in combination with detergent substances - can impair the skin barrier (Diepgen, 2003) if skin care and protection measures are not properly applied to the hands. Consequently, the combination of handling hair cosmetics, including hair colors, and inappropriate protective and restorative measures, is considered the main cause of irritant damage and skin sensitization (EU-OSHA, 2014).

Correspondingly, hairdressing is considered to be commonly associated with the development of allergic contact dermatitis (ACD), (Frosch et al., 1993, Warshaw et al., 2012, Dickel et al., 2002, Bradshaw et al., 2011) predominantly with involvement of the hands. Among other chemicals the hair dye precursors *p*-phenylenediamine (PPD) and toluene-2,5-diamine (PTD) have been described as important occupational contact allergens (Valks et al., 2005, Schwensen et al., 2014) and an association with the occupation as hairdresser has been reported (Schnuch et al., 2008, Uter et al., 2014).

Hence, evaluation of the risk to hairdressers of the induction of contact allergy presented by hair dye ingredients in hair coloring products was considered necessary. For that purpose, a scientific work shop was held in January 2016 to develop a risk assessment approach for the induction of skin sensitization specifically considering the occupational exposure conditions of hairdressers.

It has been shown that the general principles of quantitative risk assessment (QRA; for instance, (WHO, 2004, ECHA, 2012a, ECETOC, 2009)) can be applied to the induction of skin sensitization. Correspondingly, a QRA for that endpoint has been developed (Robinson et al., 2000) and described in a series of papers (Felter et al., 2002, Felter et al., 2003, Gerberick et al., 2001a, Griem et al., 2003). For hair dyes, a skin sensitization QRA approach was developed for consumers (Goebel et al., 2012, Goebel et al., 2014). Accordingly, the risk assessment phases of hazard identification and dose response characterization have already been employed in a weight-of-evidence (WoE) approach to define No Expected Sensitization Induction Levels (NESIL) for the hair dye ingredients PPD, resorcinol (Goebel et al., 2012) and PTD (Goebel et al., 2014).

A “Sensitization Assessment Factor” (SAF), a factor that reflects and accommodates uncertainty, can be applied to the selected point of departure (WoE NESIL) in order to derive an acceptable level of exposure to a skin sensitizing substance (WHO, 2008). These SAFs take into account uncertainties when extrapolating from the WoE NESIL- underlying test conditions to the actual product in-use exposure conditions. Generally, SAFs are used to account for inter-individual variability in susceptibility, product matrix possibly containing other components causing skin irritation or enhanced skin penetration, and product use patterns, accounting for factors affecting human exposure, such as use frequency, occlusion and dermal integrity (reviewed in (Basketter and Safford, 2016)). For large data sets of chemicals including PPD and other aromatic amines, it has been shown that for skin sensitization applied area doses are directly comparable between mouse and man (Basketter et al., 2005, Griem et al., 2003, Schneider and Akkan, 2004, Gerberick et al., 2001b). Correspondingly, no interspecies assessment factor has been applied to extrapolate from mouse sensitization induction data generated in the local lymph node assay (LLNA) to humans.

The next phase of the QRA is quantification of the likely hairdresser hand exposure to hair dyes under typical working conditions. Hand exposure data from hairdressers are available from previous studies assessing hair dye concentrations in hand rinses: Samples were either randomly collected during a normal working day (Lind et al., 2005), or obtained after a typical working day with six hair dyeing treatments mimicked in a controlled mass-balance study (Hueber-Becker et al., 2007).

In the present paper, we compare the WoE NESILs with hairdresser hand exposure levels to provide an estimation of the potential risk of skin sensitization associated with their occupational exposure through hair coloring products. This risk assessment approach has been applied here to the common hair dye ingredients PPD, PTD and resorcinol. Particular consideration has been given to hand size and exposed hand area as well as to wet work conditions and hand protection.

Methods

Hairdresser occupational exposure

Data on the occupational skin exposure of hairdressers to hair dyes, based on the assessment of hand rinses, were available from two publications (referred to as 1A and 1B):

1 A) A mass balance study using hair dye products spiked with [^{14}C]-labelled PPD was performed by Hueber-Becker et al. (Hueber-Becker et al., 2007). A brief summary of the experimental approach is provided below where the focus is on dermal exposure relevant for skin sensitization. Exposure to PPD of 18 experienced male (3) and female (15) hairdressers was analyzed over a 6 day working period. Hair dyeing was performed on artificial training heads wearing high-density implanted human hair equivalent to human hair in both density and length. All tools and utensils used in the study corresponded to the standard equipment of professional hairdressing salons. The hair dyeing process was divided into 3 phases: (a) preparation of the dye, actual hair dyeing and cleaning of used tools, (b) rinsing, shampooing, conditioning and superficial drying, and (c) cutting, drying (electrical dryer) and brushing. Each task was performed six times/working day. The hair dye tint used contained PPD spiked with [^{14}C]-labelled PPD and the couplers resorcinol and m-aminophenol corresponding to a marketed hair dye product.

Phase A: Prior to application, the hairdresser mixed the [^{14}C]-PPD containing tint (bare hands) with the developer (containing 6% hydrogen peroxide) in the mixing bowl with the application brush to produce the finished oxidative hair dye product containing 2% PPD. Then the hairdresser (wearing gloves) applied the hair dye mixture using a brush and comb within 15–20 min. The exposure time was 30 min to enable the dye development, i.e. the coupling reaction of PPD and the couplers inside the hair shaft.

146 *Phase B:* After dye development the hair was rinsed (wearing gloves) with a hand shower
147 and warm water. The hair was then washed twice using approximately 20–30 ml of a
148 commercial shampoo and warm water and rinsed until the water was nearly colorless.
149 Then a conditioner was applied and worked into the hair (bare hands) for about 5 min and
150 the hair was combed, rinsed and dried.

151 *Phase C:* The dried hair was cut (bare hands) and the hair was modelled using an electric
152 hair dryer, brush and clips. The style of hair cutting and modelling was chosen by the
153 individual hairdresser.

154 At the beginning and the end of each phase hands were washed thoroughly twice with a
155 water/soap solution and the hand rinse samples were collected. Urine was collected over
156 the intervals 0-12, 12-24 and 24-48 h to determine total excretion. Radioactivity in the
157 hand rinse, urine samples and on used gloves (following extraction with 70%
158 ethanol/KOH) was determined by Liquid Scintillation counting.

159 **1B)** Occupational skin exposure to permanent hair dye compounds was assessed in 33
160 hairdressers using a hand rinse method (Lind et al., 2005). A brief summary of the
161 experimental approach is provided below, with the focus being on skin exposure to PPD,
162 PTD and resorcinol. The 33 hairdressers (20 females; 13 males) were randomly recruited
163 as volunteers in 9 randomly selected salons in Stockholm, Sweden. Each hairdresser
164 participated in the study during hair dyeing of only one customer. Nine of the hairdressers
165 had performed hair dyeing previously during the same day. Hand rinse samples were
166 collected during normal working hours before the start of hair dyeing, after application of
167 the hair dye product and after cutting the newly dyed hair. Separate samples were taken
168 for the dominant and the serving hands. The dominant hand was defined as the hand
169 holding the application tool (e.g. scissors, comb) while the other hand was referred to as
170 the serving hand. Individual hands were rinsed vigorously for 2 min in a polyethene bag

containing 50 ml rinsing solution (0.2 M ascorbic acid in borate buffer with 10% ethanol).

All samples were analysed by high pressure liquid chromatography (HPLC) using standard solutions of PPD, PTD and resorcinol in buffer containing 10% ethanol and 0.2 M ascorbic acid. Usage of gloves was different during the subsequent phases of the hair dyeing process: 4/33 hairdressers used gloves while mixing the hair dye tint with the developer; 11/12 during application of the hair dye product for root coloration of grey hair; 4/16 for coloration of strands, and 8/31 for rinsing and washing.

Skin rinse and skin exposure

Dermal absorption experiments under hair dye use conditions were conducted following OECD guideline 428 (OECD, 2004a, OECD, 2004b). Data were published and the articles contain descriptions of the experimental details used for assessment of PPD (Hueber-Becker et al., 2004), PTD (SCCS, 2012), and resorcinol (SCCS, 2010). In brief, human or pig skin samples were placed as a barrier between the two halves of the diffusion cell, the dermal side of the skin was exposed to receptor fluid representing the systemic compartment, and the skin surface remained air exposed. After mixing an equal amount of the hair dye formulation (containing an adequate reaction partner) with developer, the mixture was spread evenly over the surface of the skin samples. The final formulations contained 2% PPD; 1.25% resorcinol; and four increasing concentrations of PTD sulphate (0.25, 0.8, 2.4, 7.2%), respectively, each individually spiked with the corresponding [^{14}C]-ring-radiolabeled hair dye precursor. Details of dosing are provided in Table 2. After 30 min, the formulation was removed from the skin surface by washing with water and shampoo. After a minimum of 24 h experiments were terminated. The radioactive content of all samples i.e. skin rinse and skin exposure (referred to as Measured Exposure Level [MEL] equivalent to the sum of the amounts recovered in SC, epidermis, dermis and receptor fluid) was measured by scintillation counting. Mass balance was calculated relative to the actual administered dose of [^{14}C]-labelled hair dye precursor and only

individual diffusion cells with a recovery of $100 \pm 10\%$ were considered to be evaluable.

Differences in the levels of hair dye precursors found in the skin rinses compared with the levels found for skin exposure (MEL) are expressed as factor skin rinse/MEL.

Hand and palm surface area

Data on hand surface area and on the palm area including fingers are available from Lee and coworkers (Lee et al., 2007). In brief, 65 Korean (Chinese) adults (34 males, 31 females) with an age range of between 20 and 63 years participated in the study. The hand surface area was measured directly using an alginate method by coating the skin of the hand and using the imprint to calculate the surface area. The surface area of the hand was calculated as the mean surface area of both the right and the left hand including bottom and back. For calculation of the palm surface area, the finger surface area was divided into bottom and top based on a mid line between the top and bottom of the finger on a side view. That means, the palm includes the under-half of the sides of the hand and fingers (see table 3).



Dotted lines indicate the middle line to divide the skin surface into the top and the bottom of hand and fingers, taken from (Lee et al., 2007).

Skin sensitization induction threshold

Derivation of the induction threshold for skin sensitization to hair dye precursors has been described in detail previously (Goebel et al., 2012, Goebel et al., 2014). Briefly, the

identification of the induction threshold of a given chemical is defined via a no effect level considering relevant animal and human data in a weight-of-evidence (WoE) approach referred to as the WoE NESIL (No Expected Sensitization Induction Level).

Results

Estimation of occupational hand exposure of hairdressers to hair dye precursors

The concentration of the hair dye precursors in hand rinses of hairdressers were used to assess occupational skin exposure based on data from Hueber-Becker et al. (Hueber-Becker et al., 2007) (see table 1A), and Lind et al. (Lind et al., 2005) (see table 1B).

The data in table 1A are based on a mass-balance study using radio-labeled [^{14}C]-PPD reflecting a working day with the maximal number of hair dyeing services possible and considering normal working habits regarding the use of gloves. The applied hair dye product contained 2% PPD, the maximally allowed use concentration in the EU. Hand exposure was maximized by applying the conditioner with bare hands (phase 2) comparatively to actual hair dye product application recommendations and normal working habits. The mean PPD exposure per working day (hand rinse) of all 18 hairdressers was $194 \pm 49 \mu\text{g}$ and estimated mean residues remaining on gloves were $8720 \pm 2410 \mu\text{g}$.

The data in table 1B for PPD, PTD and resorcinol are based on HPLC analyses of hand rinses collected from 33 hairdressers. For all three hair dye precursors highest exposure was observed in hand rinses after application of the hair dye with mean values for both hands (dominant plus serving hand) of 31.8, 35.4 and 95.2 μg for PTD, resorcinol and PPD, respectively. Maximal exposure values found in the study were 136.8, 141.4 and 192.2 μg , for PTD, resorcinol and PPD, respectively (see table 1B).

Estimation of skin exposure from hand skin rinse samples

Relevant skin exposure of hairdresser hand rinse samples was estimated using the relation skin rinse/MEL (see table 2). That is the relation of concentrations in the 30 min skin rinse (surface excess) and concentrations remaining on and in the skin after rinsing (MEL). Data from publicly available *in vitro* dermal absorption experiments applying oxidative hair dyes under realistic use conditions were used (Hueber-Becker et al., 2004, SCCS, 2012, SCCS, 2010). Table 2 shows the relationship between applied dose, the skin rinse fraction (surface excess) and the MEL for PPD, PTD and resorcinol after 30 min exposure. The minimal factor for skin rinse/MEL is 15 and the maximal factor is 79.

Estimation of the exposed hand area

For occupational exposure assessment of hairdressers, estimation of the hand surface area (HSA), and specifically the hand surface area exposed during the three phases of the hair dyeing procedure, is critical (see table 3). The SCCS notes of guidance refer to 860 cm² for the surface of both hands equivalent to 430 cm² per hand (SCCS, 2016). A smaller hand surface area reported from a Korean study with an average of 392 cm² per female hand (Lee et al., 2007) was considered to provide a conservative estimate to account for ethnic and sex differences among hairdressers. Subsequent determination of the palm surface area, including fingers, in that study revealed an average of 189 cm² for females and 217 cm² for males. Since during hair dyeing, particularly during phase C (cutting and drying generally performed without gloves) the entire palm surface area is not in touch with remaining hair dye product, an exposed area of 50 cm² per hand was considered to provide a conservative adjustment accounting for the expected uneven distribution during that phase. Considering both hands of a hairdresser the total exposed area is equivalent to an average of 100 cm².

Comparison of estimated hairdresser hand exposure and skin sensitization**induction thresholds**

Table 4 displays the estimated maximum levels of hairdresser hands exposure per unit area for PPD, PTD and resorcinol, and the corresponding skin sensitization induction threshold information. Column 2 provides the details how estimation of the maximal hairdresser exposure per unit area of skin is based on a) hand rinse concentrations per working day (taken from tables 1A and B), b) adjustment to relevant skin exposure concentrations by applying a factor of 0.1 to account for the relation skin rinse/MEL (see table 2) and c) adjustment for wet working conditions with increased skin hydration possibly leading to increased epidermal exposure and irritation by applying a factor of 10 (WHO, 2008). For both hands of a hairdresser the exposed area of 100 cm² (column 3) has been derived considering uneven exposure of the palm area including fingers. This included exposure during the different working phases of the hair coloring process as well as ethnic and gender differences (see table 3). Based on the data provided in columns 2c and 3 the PPD exposure of the hairdressers' hands per unit area was calculated to be 194 µg/100 cm² considered equivalent to 2 µg/cm² (column 4). The corresponding exposure doses per unit area for PTD and resorcinol are in each case 1.4 µg/cm².

Skin sensitization induction thresholds are presented in column 5: For PPD, a concentration of 27.5 µg/cm² has been previously defined as the weight-of-evidence (WoE)-NESIL based on six Local Lymph Node Assay (LLNA) data sets and two human data sets (Goebel et al., 2012). For resorcinol the most conservative value for the WoE NESIL of 350 µg/cm² was selected from two available LLNA datasets (Goebel et al., 2012). For PTD, a WoE NESIL of 41.3 µg/cm² has been derived for the free base (Goebel et al., 2014) based on a LLNA data set generated with the sulfate salt (Aeby et al., 2004, SCCS, 2012).

Comparison of the individual skin sensitization induction thresholds (see NESILs provided in column 5) with the exposure doses per unit area (taken from column 4) is represented as ratio in column 6. For PPD the ratio is 13.7 indicating that the hairdresser exposure is approximately 14-fold lower than the NESIL of 27.5 $\mu\text{g}/\text{cm}^2$. For PTD and resorcinol the corresponding ratios are 29.5 and 250, respectively. In column 6 the individual ratios are further divided by 5, to adjust for inter-individual variability for workers based on the recommendation by ECHA (ECHA, 2012b). As a result, estimated hairdresser hand exposure levels are 2.9 and 5.9 fold below the individual NESILs for PPD and PTD, respectively, and 50 fold below the NESIL for resorcinol.

Discussion

In principle, the skin sensitization QRA approach follows the same four fundamental steps as identified for general toxicological risk assessment: hazard identification, dose–response assessment, exposure assessment, and risk characterization. It implies that induction of skin sensitization is a threshold based phenomenon (Kimber et al., 1999, 2008), and that a level of skin exposure to a contact allergen at, or below which, the induction of skin sensitization will not occur can be defined (Basketter and Safford, 2016). It also implies that potential skin sensitizing ingredients can be used safely as long as skin exposure to them is below the NESIL defined in a WoE approach considering all available data (WHO, 2008).

For the hair dyes used in this study, derivation of the WoE NESIL has been thoroughly characterized by evaluation of all available human and animal data sets (Goebel et al., 2012, Goebel et al., 2014). Based on this understanding, skin exposure to concentrations equivalent to, or lower than, 27.5 $\mu\text{g}/\text{cm}^2$ PPD, 41.3 $\mu\text{g}/\text{cm}^2$ PTD, or 350 $\mu\text{g}/\text{cm}^2$ resorcinol,

are unlikely to result in skin sensitization (see table 4 column 5). For PPD, this is further supported by Ezendam and coworkers who derived a very similar NESIL of 17.5 $\mu\text{g}/\text{cm}^2$ by using the lower limit of benchmark dose analysis across 13 independent LLNA studies (Ezendam et al., 2013).

Assessment of exposure to a skin sensitizer is based upon an understanding of user habits, and of practice data of product use containing the substance, and may be complemented further by experimental measurement of skin exposure (WHO, 2008). In the context of this study hair dye hand exposure during a typical hairdresser's working day was used to determine the relevant occupational exposure.

For PPD, the exposure was determined by two different hand rinse measurements. In the mass-balance study (Hueber-Becker et al., 2007), 18 hairdressers used radio-labeled hair color products spiked with ^{14}C -PPD. The hair color product applied contained 2% PPD, the maximally allowed use concentration in the EU, even though marketed products for dark shades contain primary intermediate concentrations in the region of 1% (Antelmi et al., 2017, DK-EPA, 2014). The high recovery rate ($102.5 \pm 2.2\%$) showed that all radioactivity was accounted for, confirming that the mean PPD concentration of $194 \pm 62 \mu\text{g}$ determined in the hand rinse samples (see table 1A) represents a reliable experimental descriptor of the occupational hand exposure per working day. The relevance of using the value of 194 μg for hairdressers' hand exposure to PPD is further supported by the findings from Lind et al (Lind et al., 2005) using HPLC determination to analyze hand rinse values randomly obtained from hairdressers in salons in Stockholm. In that study the maximal value found for PPD exposure of 33 hairdressers was 192.2 μg , while the mean was 95.2 μg (see table 1B). For PTD and resorcinol maximal values measured were 136.8 and 141.6 μg , respectively, while mean values were 31.8 and 35.4 μg , respectively (table 1B). More recent hand rinsing data from Lind and coworkers (Lind et al., 2017) found a mean value of 71 μg for resorcinol (equivalent to 648 nmol) for all hair coloring tasks. This

corresponds well with the measured mean resorcinol value of 35.4 μg referred to in table 1B taking into account that the resorcinol determination from 2017 possibly contains other hair dye-related substances with the same retention time as resorcinol.

The next step for the risk assessment was to determine what percentage of hair dye exposure measured in the hand rinses can be considered as available for the induction of skin sensitization. For direct skin exposure to hair dyes under consumer use conditions the data in Table 2 indicate that more than 90% of the individual hair dye precursor are removed by rinsing (Hueber-Becker et al., 2004, SCCS, 2010, SCCS, 2012). The corresponding data for PPD, PTD and resorcinol are compiled in table 2. Application of a factor of 0.1 was therefore considered appropriate to derive the actual hand exposure level from the amounts measured in the hand rinse samples (see table 4, column 2b). In a hair dye exposure study with rats a total of 85 % was found to be rinsed off even when animals received three daily applications of 0.35% PPD in a hair color formulation each for five minutes followed by rinsing (White et al., 2007). Even though rat skin is known to overestimate the skin penetration of low molecular weight chemicals in humans by a factor of 2 to 3 (Simon and Maibach, 2000), the findings by White et al. (White et al., 2007) support our approach to adjust the concentrations found in the hand rinses by a factor of 0.1 to estimate the hairdresser hand exposure.

Estimation of an exposed area of 50 cm^2 per hand was driven by conservatively accounting for ethnic and sex differences among hairdressers, such as a smaller size of female hands (see table 3), based on (Lee et al., 2007), and for uneven distribution during cutting and styling with bare hands (Lind et al., 2005). Since the risk assessment for skin sensitization is based on dose per unit area of skin comparisons, the smaller the exposed hand area used, the higher the hair dye dose per unit area becomes. Consequently, using an area of 100 cm^2 for both hands is 8 times more conservative than the SCCS standard

for the hand surface area of 860 cm² commonly used for dose per unit area calculations of hand exposure (SCCS, 2016).

The next key consideration for the risk assessment for hairdressers is wet work that has been shown to occur for an average duration of slightly above 2 hours per working day (Kralj et al., 2011). The continuous wetting and drying of the hands during several hairdressing procedures, as well as contact with a wide range of cosmetic products, has been considered to increase the removal of natural lipids causing the skin to dry out, flake, split, and crack (Behroozy and Keegel, 2014). Consequently, up to 70% of hairdressers have been reported to experience work-related skin damage at some point during their career (English, 2004). Since this may be associated with increased irritant inflammation (such as irritant contact dermatitis) it is also considered to increase the susceptibility to the induction of skin sensitization (Caroe et al., 2017). Furthermore, elicitation responses to nickel and to the preservative methyldibromo glutaronitrile were found to occur at up to 10 fold lower doses in the presence of concomitant irritancy (Allenby and Basketter, 1993, Menne and Calvin, 1993, Pedersen et al., 2004). Consequently, a factor of 10 was applied to account for the higher risk of skin barrier damage and/or irritant inflammation related to wet work conditions (table 4 column 2c), in line with risk assessment approaches for skin sensitization to chemicals (WHO, 2008).

From a toxicological risk assessment perspective, the described pattern of occupational exposure has been adequately considered in the hand exposure studies by the teams of Hueber-Becker and Lind, respectively (Hueber-Becker et al., 2007, Lind et al., 2005), and the derived hand exposure levels for PPD, PTD and resorcinol represent reliable descriptors that adequately address the daily exposure frequency. Compared with hair dye product formulations, experimental vehicle matrices used in the LLNA for the hair dye ingredients (mainly acetone olive oil, dimethyl sulfoxide, and dimethylformamide) are known to exhibit penetration enhancing and irritating effects (Woodford and Barry, 1986,

Lashmar et al., 1989, Tsai et al., 2001). Consequently, SAFs accounting for uncertainties regarding different matrix effects and/or patterns of product use, summarized in (Goebel et al., 2012) other than the SAF of 10 for wet work conditions were not considered relevant.

Uncertainties accounting for inter-individual variability regarding the induction of skin sensitization in the general population has been recently reviewed in (Basketter and Safford, 2016), concluding that the default value of 10 is adequate, in line with our previous assumptions for consumers of hair color products (Goebel et al., 2012). The uncertainty factor of 10 is also recommended for other threshold-based toxicological endpoints such as systemic toxicity effects by the European Chemical Agency (ECHA, 2012b). For professionals, ECHA recommends a default factor of 5, considering a decreased risk for workers compared with elderly or diseased subpopulations (ECHA, 2012b, Dankovic et al., 2015). Although the individual susceptibility differences for the induction of skin sensitization are toxicologically covered by that default factor, it should be noted that this may not be the case for elicitation. The latter is also affected by differences in the induction dose (Hostynek and Maibach, 2004) and thus the default value may not be adequate.

Comparing the NESIL with the maximal hairdresser hand exposure values including the adjustments for wet work conditions, hand size and exposed hand area, indicated ratios >10, >20 and >250 for PPD, PTD and resorcinol, respectively. Considering a factor of 5 for inter-individual variability for professionals the comparison of the NESIL and the hand exposure for PPD and PTD are within the same order of magnitude compared to a factor of >50 for resorcinol. The limited distance between hand exposure and the threshold for allergy induction for PPD and PTD indicates that hand protection and skin care are of major importance to keep skin exposures as low as possible. More explicitly, PPD residues remaining on gloves following application/rinsing of the hair color product are more than 40-fold higher than the hand residues when gloves were used (see table 1A).

Even though training for hairdressers including guidance regarding protective measures and skin care had led to certain desirable workplace behaviors, it has been reported to not always have resulted in health risk awareness (Bradshaw et al., 2011). Correspondingly, questionnaire data from 295 hairdressers demonstrate that no gloves were used by 22% when dyeing hair strands (highlights) and that 36% used gloves only occasionally (Johansson et al., 2015). Among the no-glove users for highlights, hand exposure was found to differ more than 3-fold depending on personal skills (Lind et al., 2017). In our view the limited application of preventive hand protection for hair color applications such as highlights may lead to allergy induction and is consistent with the high prevalence of hair dye allergy in hairdressers (see table 5). It has been shown that preventive measures are rather used as a consequence of symptom development, rather than for prevention prior to their development (Perkins and Farrow, 2005).

This is in line with an increased prevalence of PPD patch test positive hairdressers compared with sex and age-matched control dermatological subjects by a factor of 3 to 7 fold (Malvestio et al., 2011, Schwensen et al., 2014, Schubert et al., 2018). Compared with the prevalence of positive patch tests to hair dye precursors in consumers, it appears that it may be somewhat lower in most of the periods studied (see Table 5 for details). In some studies this may reflect a selection bias, as hairdressers are “routinely” tested with the hairdressers’ series while a stronger suspicion of hair dye allergy is needed for the application of the series in clients/consumers (Uter et al., 2003). However, how non-occupational risk behaviors of hairdressers regarding the more frequent personal use of hair color products and of black henna tattoos (Bregnhøj et al., 2011, Hansen et al., 2010) affect the risk for their contact dermatitis has not yet been assessed. Since black henna tattooing has been described as a relevant risk factor for contact allergy to PPD (Diepgen et al., 2016) information about risks arising from personal behaviors is considered an important part of the education of apprentices.

Based on the knowledge that hairdressers are a high-risk group for occupational hand eczema related to contact dermatitis as compared with other occupational groups (Dickel et al., 2001, Skoet et al., 2004, Schwensen et al., 2013), education and training efforts to improve their health risk awareness and to consequently apply preventive measures to avoid exposure have been established by regulators, occupational organizations and industry (Sonsmann et al., 2011, Safehair, BAuA, 2008, HSE, 2006, IKW, 2017). Recently, the importance of training regarding the use of gloves during hair dyeing in a real work situation has been demonstrated: correction of improper glove use led to a very effective reduction of the daily exposure to PPD and PTD (Geens et al., 2016). Consequently, adequate skin protection and proper training instructions for the use of adequate, disposable gloves is most likely the most effective way to minimize the risk of contact allergy resulting from hair dyeing (Antelmi et al., 2015, Hougaard et al., 2015, Lind et al., 2017, Oreskov et al., 2015).

In summary, comparison of the allergy induction threshold and the hairdresser hand exposure, including the application of SAFs to account for wet work and inter-individual differences among workers, indicates that maximal hand exposures to PPD and PTD were 2.7 and 5.9 fold below the NESIL. It confirms the current observation that occupational exposure to PPD and PTD may lead to the induction of skin sensitization if skin protection and skin care are not thoroughly followed.

We conclude that the awareness of health risks associated with occupational exposure to wet work and to potentially sensitizing cosmetic chemicals, as well as of the importance of appropriate protective measures need to be further increased during hairdresser education and trainings.

Conflict of interest statement

Carsten Goebel, Anne Fuchs, Kordula Schlotmann and Maya Krasteva are employees of companies selling cosmetic products including hair colorants. The hair dye ingredients studied in this paper are currently used in commercial products marketed by these companies. A one day workshop was organized and sponsored by these companies and Thomas Diepgen, Brunhilde Blömeke, Anthony Gaspari, Axel Schnuch and Ian Kimber were invited as consultants. The authors alone are responsible for the content and writing of the paper.

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Legend to figure 1

Workflow of quantitative risk assessment for skin sensitization of hairdressers to hair dyes considering their occupational exposure

Table 1A: Estimation of occupational hand exposure of hairdressers to *p*-phenylenediamine (PPD) per working day¹

Phases of hair dyeing process	Phase A ² Dye preparation and hair dyeing with gloves	Phase B ² Shampooing/rinsing with gloves ; Conditioning w/o gloves	Phase C ² Cutting/drying w/o gloves	Mean (A+B+C)
Hand residues (dominant and serving hand) in hand rinse samples	5 ± 2	61 ± 13	128 ± 47	194 ± 49
Residue on gloves³	7620 ± 2380	1100 ± 380	-	8720 ± 2410

¹ reference: (Hueber-Becker et al., 2007), ²Data are presented in µg [¹⁴C]-PPD_{eq}/per working day including 6 complete hair dyeing procedures (± SD) consisting of phases A, B, and C per hairdresser (n=18) participating in a mass balance study under controlled conditions. The hair color product used contained 2% PPD after mixing with the developer, equivalent to the maximally allowed use concentration in the European Union. ³considering single procedure. w/o, without.

Table 1B: Estimation of occupational hand exposure of hairdressers by residues in hand rinse samples randomly taken after hair dye application during normal working day¹

Hair dye precursor	Positive samples ² dominant/serving hand	Dominant hand ³ mean and (range)	Serving hand ³ mean and (range)	Mean (dominant + serving hand)	Maximal value (dominant + serving hand)
PPD	4/4	49.1 (2.3 - 101.5)	46.1 (3.9 - 90.7)	95.2	192.2
PTD	12/11	14.4 (2.3 - 46.3)	17.4 (1.6 - 90.5)	31.8	136.8
Resorcinol	21/21	20.4 (3.3 - 56.5)	15.0 (2.6 - 85.1)	35.4	141.6

¹ reference: (Lind et al., 2005); ²randomly obtained from hairdressers (n=33) during normal working hours at nine different hairdressing salons in Stockholm, Sweden, using an HPLC method; ³data are presented in µg (for conversion of data from nmol to µg the molecular weight of the free base of the hair dye was used)

Table 2: Distribution of hair dye precursors between skin rinses and skin exposure after application of an oxidative hair dye product

Hair dye precursor	Skin source	Applied dose ¹ [$\mu\text{g}/\text{cm}^2$]	Skin rinse ² (surface excess rinsed off) [%]	Measured Exposure Level (MEL ³ ; = skin exposure after rinsing in SC+ epidermis + dermis + receptor fluid) [%]	Factor Skin rinse/MEL	Reference
PPD	human	445.6 $\pm$ 39.2 (equivalent to 2.0%)	98.8 $\pm$ 5.9	3.7 $\pm$ 1.4	26	(Hueber-Becker et al., 2004)
	pig	423.0 $\pm$ 31.4 (equivalent to 2.0%)	92.4 $\pm$ 3.0	5.1 $\pm$ 1.6	18	
PTD	human	60.9 $\pm$ 9.3 (equivalent to 0.25%)	92.69 $\pm$ 3.77	6.2 $\pm$ 1.8	15	(SCCS, 2012)
	human	181.5 $\pm$ 31.2 (equivalent to 0.8%)	94.25 $\pm$ 3.13	5.9 $\pm$ 1.4	16	
	human	566.9 $\pm$ 83.7 (equivalent to 2.4%)	96.62 $\pm$ 3.73	5.7 $\pm$ 1.4	17	
	human	1574.4 $\pm$ 303.6 (equivalent to 7.2%)	94.19 $\pm$ 3.79	5.6 $\pm$ 1.5	17	
Resorcinol	human	266.3 $\pm$ 13.3 (equivalent to 1.25%)	95.35 $\pm$ 6.09	1.2 $\pm$ 0.5	79	(SCCS, 2010)

¹Applied dose of [¹⁴C]-labeled PPD, PTD sulfate or resorcinol in μg equivalents/ cm^2 in approximately 20 mg/cm^2 hair dye product to human skin and/or pig ear skin samples; percent of applied radioactivity ²in skin rinses after 30 min or ³ in stratum corneum (SC) and bioavailable compartments at 24 h after administration

Table 3: Single hand and palm surface areas

Source	Ethnic group	Total body surface area [cm ²]	Hand surface area [cm ²]	Palm surface area including fingers [cm ²]	Estimated exposed surface area of hairdresser hand during hair dyeing procedure ³ [cm ²]
(Lee et al., 2007) ¹	Chinese (Korean)	Male: 18339 ± 1938 Female: 16452 ± 1960	448 ± 41 392 ± 41	217 ± 19 189 ± 21	50
(SCCS, 2016) ²	European	17500	430	not determined	

¹ based on 65 adults, 34 males and 31 females; ²prediction for the European population generated using probabilistic analysis;

³considering assumptions for uneven exposure to hair dye product during habits and practices of hair dyeing procedure including preparation and application, shampooing, rinsing and conditioning, and cutting and drying

Table 4: Comparison of hairdresser maximal hand exposure and skin sensitization induction threshold

1	2a	2b	2c	3	4	5	6	7
Hair dye precursor	Estimated exposure dose of both hands [μg]			Estimated exposed surface area of both hands ⁴ [cm ²]	Exposure dose /unit area [μg/cm ²]	Skin sensitization induction threshold (NESIL) ⁵ [μg/cm ²]	Ratio [NESIL/exposure dose]	Ratio adjusted for inter-individual variability among workers ⁶ [ratio/5]
	Working day ¹	Adjustment for overestimation by rinsing ²	Adjustment for wet working conditions ³					
PPD	194 ± 49	19.4	194	100	2	27.5	13.7	2.7
PTD	137	13.7	137	100	1.4	41.3	29.5	5.9
Resorcinol	142	14.2	142	100	1.4	350	250	50

¹values taken from table 1A for PPD (mean + SD) and 1B for PTD and resorcinol (maximal values rounded up); ²application of factor of 0.1 to account for relation between skin rinse and MEL based on compilation in table 2; ³factor of 10 for wet working conditions (WHO, 2008); ⁴adjusted for uneven hand exposure during hair dyeing procedure (rounded up for PPD), see table 3; ⁵NESIL for free base of hair dye based on WoE derivation from human and LLNA data taken from (Goebel et al., 2012, Goebel et al., 2014); ⁶default factor of 5 based on ECHA recommendation for inter-individual variability for workers (ECHA, 2012b)

Table 5: Prevalence of positive patch-test reactions to selected hair dyes (standard and hairdressers

series) in hairdressers compared to other patient populations

Source	Study country	Study period	Hair dye precursor	Hairdressers		Non hairdressers		Statistics	
				Number tested	[%] patch test positive	Number tested	[%] patch test positive	Crude odds ratio	95% confidence interval
(Malvestio et al., 2011)	Italy	1997-2004	PPD	136 females	14.7	2206 females (internal control group of office workers)	3.0	6.58	3.76-11.5
				13 males	15.4	1024 males (internal control group of office workers)	1.5	22.3	4.18-119
(Schwensen et al., 2014)	Denmark	2002-2011	PPD	399	9.0	1995 (sex and age-matched unselected)	1.2	9.86	5.64 – 17.22
(Frosch et al., 1993)	9 European centres	1988-1991	PPD	809	14.83	104 (clients)	19.2	n.a.	
			PTD		7.55		8.7		
			Resorcinol		0.56		1.9		
(Uter et al., 2003)	IVDK	1995-2002	PPD	884	18.7	1217 (female clients)	15.4	n.a.	
			PTD	(females)	22.3		13.9		
(Uter et al., 2007)	IVDK	2003-2004	PPD	287 (females)	18.1	394 (female clients)	20.3	n.a.	
		2005-2006	PTD	480 (females)	19.6	780 (female clients)	22.6		
(Uter et al., 2014)	IVDK	2007-2012	PPD	824	20.4	2067 (female clients)	27.0	n.a.	
			PTD	(females)	21.2		26.3		
(Schubert et al., 2018)	IVDK	2008 - 2013	PPD	232	18.5	4082 (IVDK PPD tested patients)	5.6	2.1	1.3 - 3.2

n.a. (not available from reference)

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Hand exposure study 1
*mass balance study
analyzing [^{14}C]-PPD in hand
rinsings (n=18), see table 1A*

Hand exposure study 2
*real-life HPLC-study analyzing
PPD, PTD, Resorcinol in hand
rinsings from Stockholm
salons (n=33), see table 1B*

**Skin exposure
assessment
from hand rinse**
see table 2

Adjust

**Total hand exposure
per working day**

see table 4

Adjust

**Wet work
consideration**
regarding impaired skin
barrier and skin irritation

Calculate

**Hand exposure/
unit area**

Compare

**Ratio
NESIL/exposure
dose**

Adjust

**Exposed hand
surface area
assessment**
considering unequal
distribution/ethnic and sex
differences in hand size
see table 3

**Skin sensitization induction
threshold (NESIL)**
*derived from substance-specific data for
PPD, PTD, Resorcinol*

**Inter-individual variability
among workers**