



Workshop Report

Quantitative risk assessment for skin sensitisation: Consideration of a simplified approach for hair dye ingredients

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ABSTRACT

With the availability of the local lymph node assay, and the ability to evaluate effectively the relative skin sensitizing potency of contact allergens, a model for quantitative-risk-assessment (QRA) has been developed. This QRA process comprises: (a) determination of a no-expected-sensitisation-induction-level (NESIL), (b) incorporation of sensitization-assessment-factors (SAFs) reflecting variations between subjects, product use patterns and matrices, and (c) estimation of consumer-exposure-level (CEL). Based on these elements an acceptable-exposure-level (AEL) can be calculated by dividing the NESIL of the product by individual SAFs. Finally, the AEL is compared with the CEL to judge about risks to human health.

We propose a simplified approach to risk assessment of hair dye ingredients by making use of precise experimental product exposure data. This data set provides firmly established dose/unit area concentrations under relevant consumer use conditions referred to as the measured-exposure-level (MEL). For that reason a direct comparison is possible between the NESIL with the MEL as a proof-of-concept quantification of the risk of skin sensitization. This is illustrated here by reference to two specific hair dye ingredients *p*-phenylenediamine and resorcinol. Comparison of these robust and toxicologically relevant values is therefore considered an improvement versus a hazard-based classification of hair dye ingredients.

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Abbreviations: AEL, acceptable exposure level; AOO, acetone olive oil; CEL, consumer exposure level; DMF, dimethylformamide; DMSO, dimethylsulfoxide; EC3, effective concentration of the test substance required to produce a three-fold increase in the stimulation index compared to vehicle-treated control; EU, European Union; HMT, human maximisation test; HRIPT, human repeated insult patch test; LLNA, local lymph node assay; MEL, measured exposure level; NESIL, no expected sensitization induction level; OECD, organisation for economic co-operation and development; PPD, *para*-phenylenediamine; QRA, quantitative risk assessment; SAF, sensitisation assessment factor; SC, stratum corneum; SCCP, scientific committee for consumer products; SCCS, scientific committee for consumer safety; SI, stimulation index; SLS, sodium lauryl sulfate; WoE, weight-of-evidence.

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1. Introduction

The risks of allergic contact dermatitis associated with the use of hair colorants is well established (reviewed in Thyssen and White, 2008; Krasteva et al., 2009). For this reason hair colorants have been under regulatory scrutiny for many years and both regulators and manufacturers have introduced measures to increase allergy risk awareness of consumers, and to minimize such risks through provision of maximal use concentrations, specific warning labels and precise usage and safety instructions (Schlatter et al., 2007; Krasteva et al., 2010).

In addition, extensive research has sought to improve hair coloring technology, with a particular focus on developing products

with reduced sensitizing potential. However, new colorant technologies that will deliver such benefits have yet to emerge (Schlatte et al., 2007).

Regulatory categorization of skin sensitization potential of hair dye ingredients is currently based on hazard potency ranking into extreme, strong and moderate sensitizers (SCCP, 2006a; Kimber et al., 2003). This is based on information derived from sensitization tests such as Guinea pig assays and/or the local lymph node assay (LLNA). Following the SCCP memorandum (SCCP, 2006a) the EU Commission chose to establish a working group charged with improving consumer protection particularly regarding the assessment and management of risks associated with contact allergy. Within that framework, the EU commission states that threshold values need to be determined for each skin sensitizer of high concern in order to enable appropriate measures to reduce the exposure of consumers (EU commission communication, 2007). In order to determine threshold values for skin sensitizers to reduce the exposure of consumers, application of quantitative risk assessment (QRA) is considered an adequate approach translating hazard-based categorizations into assessments based on actual consumer exposure.

In general, QRA estimates safe exposure levels for individual chemicals under well-defined exposure conditions. The QRA process for skin sensitization has been described in detail elsewhere (Basketter, 2008a; Gerberick et al., 2001a; Felter et al., 2003; Griem et al., 2003; Api et al., 2008; Corea et al., 2006).

Practically, the first component for QRA for skin sensitization is the identification of the induction potency of a given chemical 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). For many hair dye ingredients this induction potency information is publicly available and based largely on data deriving from the LLNA (SCCP/SCCS, Link to opinions on individual hair dye ingredients). Once the NESIL is derived, based on the relevant experimental data sets, the next step is extrapolation to the actual human exposure conditions. This is achieved usually by the incorporation of Sensitisation Assessment Factors (SAFs), also referred to as uncertainty factors. These SAFs take into account uncertainties when extrapolating from the WoE NESIL-underlying test conditions to the actual product in-use exposure conditions. Usually, they cover areas of uncertainty by values from 1 to 10 for: (a) inter-individual variability, accounting for susceptibility differences caused, among others, by age, sex, race, genetic factors and compromised skin, (b) product matrix, i.e., other components causing skin irritation or enhanced skin penetration, and (c) product use patterns, accounting for factors affecting human exposure, such as use frequency, occlusion and dermal integrity. The scientific basis for their use in QRA for the induction of allergic contact dermatitis has been published (Felter et al., 2002). These SAFs have been applied e.g. in QRAs for nickel, chromium and cobalt as well as for preservatives and fragrance ingredients (Gerberick et al., 2001a; Basketter et al., 2003; Basketter et al., 2008b; Api et al., 2008).

The second component of the QRA is the quantification of the actual consumer exposure (Consumer Exposure Level, CEL, expressed as dose per unit area). Hair coloring conditions are relatively uniform across all available hair dye products, i.e. application to the hair for a contact time of approximately 30 min followed by rinsing with water and shampoo (Brody and Bruns, 1968; Hueber-Becker et al., 2004; Aebly et al., 2009). Accordingly, the EU regulation considers hair dyeing as a typical exposure scenario (SCCS, 2010a) to be assessed in dermal absorption studies (OECD, 2004a; SCCS, 2010b) for individual hair dye ingredients to distinguish the bioavailable dose relevant for systemic risk assessment from the applied dose (see SCCP/SCCS, Link to opinions on individual hair dye ingredients). This approach has been applied

to determine the relevant actual exposure in the context of contact dermatitis to hair dyes and is referred to as the measured exposure level (MEL, Goebel et al., 2010). As the applied hair dye formulation is washed off the skin surface after 30 min by removing the surface excess whereas the experimental measurements continue for 24 h, the MEL represents the actual dose theoretically available for allergy induction under hair dyeing conditions, i.e. the amounts on/in the skin surface (i.e. Stratum corneum (SC)) and the bioavailable amounts (i.e. living skin and system).

In this paper, we introduce a direct comparison of the NESIL with the MEL as a simplified approach that allows an appropriate estimation of the potential skin sensitization risk associated with hair colorants. This concept has been applied here to the common hair dye ingredients *para*-phenylenediamine (PPD) and resorcinol as proof of concept exercises to determine whether this simplified approach is appropriate for such products.

2. Methods

2.1. Local lymph node assay (LLNA)

LLNAs considered in this paper were performed as described for PPD (Warbrick et al., 1999; White et al., 2006; SCCP, 2006b; Aebly et al., 2009), resorcinol (SCCS, 2010c; Basketter et al., 2007b) and in accordance with OECD guideline 429 (OECD, 2002).

Briefly, the chemical was dissolved in the indicated vehicle as detailed in Table 1a for PPD and in Table 1b for resorcinol. Each dosing group contained at least 4 female CBA/J mice. A dose volume of 25 μ l was topically applied to the dorsal surface of both ears once daily for 3 consecutive days. In each experiment three to five increasing concentrations were tested. Control animals received the vehicle alone. Five days following the initiation of treatment, all animals were injected with tritiated thymidine. After approximately 5 h, they were killed and the auricular lymph nodes excised. The nodes from each group were pooled, a suspension of auricular lymph node cells prepared, and proliferation of these cells measured using β -scintillation counting. The results were used to calculate the Stimulation Index (SI) for proliferation relative to the concurrent vehicle control for each dose. The estimated concentration required to induce a SI of three (EC3 value) relative to concurrent vehicle-treated controls was calculated by interpolating between two points on the SI axis immediately above and below the EC 3 as described (OECD Guideline 429).

2.2. Dermal absorption (skin binding)

Dermal absorption experiments under hair dye use conditions considered in this paper were conducted following OECD guideline 428 (OECD, 2004a; OECD, 2004b). Experimental details are described elsewhere for PPD (Hueber-Becker et al., 2004; Goebel et al., 2010; SCCP, 2006b) and for resorcinol (SCCS, 2010c). 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 on the surface of the skin samples. The final formulation contained 2% PPD or 1.25% resorcinol, spiked with radiolabeled material and dosing details are provided in Table 3. After 30 min, the formulation was removed from the skin surface by washing with water and shampoo. After a minimum of 24 h the experiments were terminated. All samples i.e. surface excess, skin (SC, epidermis, dermis) and receptor fluid were subjected to determination of radioactivity by scintillation counting. Mass balance was calcu-

Table 1(a) Collation of Local Lymph Node Assay data for *p*-phenylenediamine. (b) Collation of Local Lymph Node Assay data for resorcinol.

Reference Vehicle Applied dose [%]	Warbrick et al. (1999) AOO Stimulation index ^a	White et al. (2006) AOO Stimulation index	SCCP (2006b) AOO Stimulation index	Aeby et al. (2009) DMSO Stimulation index
(a)				
0.01		0.7	0.9	
0.025		1	1.5	
0.05	2.2	1.6	1.3	2.6
0.1	4.2	2.6	1.9	3.2
0.25	13.7	5.6	7.1	5.5
0.5	20.8	9.5		
0.75				11.2
1	25.3	9.4		
1.25			16.1	
2.5				16.8
EC3 value [%]	0.07	0.15	0.13	0.09 ^b
Reference Vehicle Applied dose [%]		Basketter et al. (2007b) AOO Stimulation index ^c	SCCS (2010c) DMF Stimulation index	
(b)				
0.1		0.9		1.6
0.25		0.5		
0.5		1.2		2.9
1		1.8		2
2.5		2.3	3.8	
5		2.6	4.1	3.5
10		6.3	4	
25		10.1	3.5	5.7
50		12.5	3.3	
EC3 value [%]		5.5	nc	1.4

AOO, acetone olive oil, DMF, dimethylformamid, DMSO, dimethylsulfoxid, EC3 value, concentration of a chemical necessary to achieve a threefold stimulation of proliferation in lymph nodes draining the site of chemical application, Stimulation index, proliferation in local lymph node of test chemical treated ears divided by proliferation in local lymph node of vehicle treated control ears, nc, not calculated.

^a Mean of four independent experiments.

^b Calculated by non linear regression with curve fit.

^c Based on combination of two experiments using historical control value for vehicle control.

lated relative to the actual administered dose of [14C]-labelled hair dye precursor and only individual diffusion cells with a recovery of $100 \pm 10\%$ were considered valid.

3. Results

3.1. No effect exposure levels for the induction of skin sensitization: LLNA-based EC3 values

For PPD, a summary of relevant published LLNA data is presented in Table 1a. The average of the 6 EC3 values presented indicates a mean EC3 value of 0.11%. This is in line with the EC3 derived by Basketter et al. considering only data from experiments where the vehicle acetone olive oil (AOO) was used (Basketter et al., 2007a).

To convert the reported EC3 concentrations in percent into specific dose per unit area values ($\mu\text{g}/\text{cm}^2$) the calculation is based on an exposed surface of 1 cm^2 per mouse ear (Robinson et al., 2000), and according to the standard protocol, a volume of $25 \mu\text{l}$ test solution (equivalent to 25 mg) is distributed over this surface. Correspondingly, 0.11% PPD in 25 mg test solution results in the specific dose per unit area value of $27.5 \mu\text{g}/\text{cm}^2$.

For resorcinol, a summary of relevant published data sets is compiled in Table 1b. Based on a data set using dimethylformamide (DMF) as vehicle, the EC3 value was found to be 1.4% (SCCS, 2010c). As explained above, the corresponding dose per unit area value was found to be $350 \mu\text{g}/\text{cm}^2$. When AOO was used as vehicle the EC3 value was 5.5% (equivalent to $1375 \mu\text{g}/\text{cm}^2$) derived by interpolation using the entire dose response curve from two experiments (Basketter et al., 2007b). As only two datasets were avail-

able, the most conservative value from data set 1 was selected, i.e. $350 \mu\text{g}/\text{cm}^2$.

3.2. Human no effect levels

Two published human data sets are available that indicate the quantitative relation of applied doses of PPD and the induction of skin sensitization in volunteers in the human maximisation test (HMT) (Kligman, 1966) and in the human repeat insult patch test (HRIPT) (Marzulli and Maibach, 1974), respectively. In both studies, treatment conditions were maximized comparatively to the standard HRIPT which consists of 9 rather than 10 allergen applications and does not include a sodium lauryl sulfate (SLS) pretreatment (McNamee et al., 2008).

In the HRIPT study, no direct information on the dose per unit area is available. Assuming that 0.1% and 0.01% are in the range of 100 and $10 \mu\text{g}/\text{cm}^2$, respectively, 11% and 7% of subjects became sensitized after 10 repeated applications compared to 53% of subjects at $1000 \mu\text{g}/\text{cm}^2$ (see Table 2b). When pretreated with SLS to enhance skin penetration/inflammation according to the HMT design, 5/24 individuals were induced by applying a single dose of $71.4 \mu\text{g}/\text{cm}^2$ (see Table 2a). Extrapolation of these results to doses where the likelihood of induction is below 5% was performed leading to doses of 6.9 and $16 \mu\text{g}/\text{cm}^2$ for the HRIPT and HMT, respectively (Schneider and Akkan, 2004).

Considering these human induction data regarding a single PPD exposure in the absence of SLS it can be roughly estimated that a dose between 10 and $100 \mu\text{g}/\text{cm}^2$ may induce skin sensitization in more sensitive individuals. A single dose is considered relevant as the exposure frequency of permanent hair dyeing is every 4–6 weeks. Within a period of 4 weeks, chemicals remaining on and

Table 2
(a) Overview of Human Maximization Test (HMT) results with *p*-phenylenediamine (PPD). (b) Overview of Human Repeat Insult Patch Test (HRIPT) results with *p*-phenylenediamine (PPD).

Dose applied in (%)	Dose converted into ($\mu\text{g}/\text{cm}^2$)	Positive reactions/total	Positive reactions in (%)
(a)			
0.1	71.4	5/24	20
1	714	17/25	68
10	7140	24/24	100
25	17875	20/20	100
Single dose applied in (%)	Single dose converted into ($\mu\text{g}/\text{cm}^2$)	Positive reactions/total	Percent of positive reactions
(b)			
0.01	10	7/97	7.2
0.1	100	11/98	11.2
1	1000	47/88	53.4

(a) For induction, PPD was applied in petrolatum under occlusion (Werbril patch) for 48 hours, 1 ml/14 cm², pretreatment with 5% aqueous sodium lauryl sulphate (SLS) for 24 hours was performed prior to induction application, for elicitation, PPD was applied in 0.4 ml/2.54 cm² petrolatum under occlusion for 48 h, reading was performed at removal of the patch and 48 h later, for calculation 1 ml was considered as 1000 mg resulting in estimation of the specific dose in $\mu\text{g}/\text{cm}^2$ by multiplying the concentration of PPD in petrolatum in percent with a factor of 1000 (modified from Kligman (1966)).

(b) For induction, PPD was applied in petrolatum with 500 mg per application and 10 repetitions within 3 weeks at the same site, 500 mg test formulation were applied on a 5 cm² patch (Johnson & Johnson Square Band Aid), resulting in estimation of the specific dose in $\mu\text{g}/\text{cm}^2$ by multiplying the concentration of PPD in petrolatum in percent with a factor of 1000, for elicitation a non irritating concentration was applied 2 weeks after the final induction treatment, i.e. 0.01 and 1% in petrolatum, respectively (modified from Marzulli and Maibach (1974)).

Table 3
Collation of skin absorption data *in vitro* considered for Measured Exposure Level (MEL) determination.

Product type	Hair dye product with 2% <i>p</i> -phenylenediamine (PPD) ^a		Hair dye product with 1.25% Resorcinol ^a
Reference	Hueber-Becker et al. (2004)	Goebel et al. (2010)	SCCS (2010c)
Type of skin	Pig	Human	Pig
Applied dose ^b [$\mu\text{g}/\text{cm}^2$]	400	400	3000
Surface excess ^c	401.1 ± 19.6	429.6 ± 29.3	2662.6 ± 70.4
Stratum Corneum (SC)	7.31 ± 3.07	5.57 ± 2.67	5.9 ± 1.8
Epidermis (E) + Dermis (D)	13.19 ± 6.15	5.65 ± 2.39	2.01 ± 1.05
Receptor Fluid (RF)	1.43 ± 0.82	4.92 ± 4.82	0.2 ± 0.12
MEL = (SC + E + D + RF)	21.93 ± 6.92	16.05 ± 6.01	0.84 ± 0.39
		6.8 ± 1.87	3.05 ± 1.13

Data presented in μg ¹⁴C-hair dye ingredient equivalent/cm² at 24 h after application of hair dye product (after mixing with developer) for 30 min.

^a Maximal allowed on-head use concentration in European Union.

^b Infinite dose levels equivalent to maximal absorption conditions for 30 min exposure period.

^c Collected at product rinsing step 30 min after application. MEL values used for QRA calculations are given in bold font.

in the SC and epidermis will be removed by continuous outward proliferation, differentiation and desquamation processes (Houben et al., 2007).

3.3. Derivation of no expected sensitization induction level (NESIL)

Based on the LLNA EC3 values from 6 different data sets for PPD a dose of 27.5 $\mu\text{g}/\text{cm}^2$ can be considered as the threshold concentration for the induction of skin sensitization. This is in line with the estimation from human data suggesting a single exposure threshold to be in the range of 10 to 100 $\mu\text{g}/\text{cm}^2$.

Consequently, there is a high likelihood to consider an applied dose of 27.5 $\mu\text{g}/\text{cm}^2$ as a threshold level for the induction of skin sensitization by a single exposure to PPD. In other words, the weight of evidence (WoE) from human and LLNA data suggests that 27.5 $\mu\text{g}/\text{cm}^2$ represents a reliable NESIL for a single exposure to PPD. This is referred to as the WoE NESIL.

In the absence of human data for resorcinol and as only two LLNA datasets were available to derive the WoE NESIL, the most conservative value was selected, i.e. 350 $\mu\text{g}/\text{cm}^2$.

3.4. Experimental exposure determination for hair dye ingredients under use conditions: measured exposure level (MEL)

The MEL can be derived from suitable dermal absorption experiments by calculating the sum of the hair dye precursor concentration on/in skin and system (SC + epidermis + dermis + receptor fluid) after an experimental period of at least 24 h. Concentrations

detected in the receptor fluid represent the amount that has reached the systemic circulation (blood). The MEL is expressed as a dose/unit area value (e.g. $\mu\text{g}/\text{cm}^2$; Goebel et al., 2010).

For PPD and for resorcinol, published skin absorption data are available (see Table 3). In both cases, exposure conditions were equivalent to a complete hair colouring process, i.e. a formulation containing maximum allowed use concentrations, i.e. 2% for PPD and 1.25% for resorcinol and relevant reaction partners was applied to the skin surface of a skin sample after mixing with hydrogen peroxide, followed by rinsing of the skin surface with water and shampoo after 30 min. After the experimental period of 24 h, the experiment was terminated and the hair dye concentration in the skin compartments (SC + epidermis + dermis + receptor fluid) was summed up to calculate the MEL. The results obtained with human skin were considered as most relevant for PPD because they are slightly above the mean value from the reported studies (Table 3) and due to the use of the most relevant species. Consequently, MEL values of 16.1 $\mu\text{g}/\text{cm}^2$ for PPD and 3.1 $\mu\text{g}/\text{cm}^2$ for resorcinol were used for comparison with the corresponding NESIL values. Fig. 1 illustrates the relation between the NESIL and the MEL for PPD (Fig. 1A) and resorcinol (Fig. 1B) on a logarithmic scale.

4. Discussion

Risk assessment in skin sensitization is based on general principles of toxicology including dose-response relationships, i.e. also for chemicals that possess the ability to cause skin sensi-

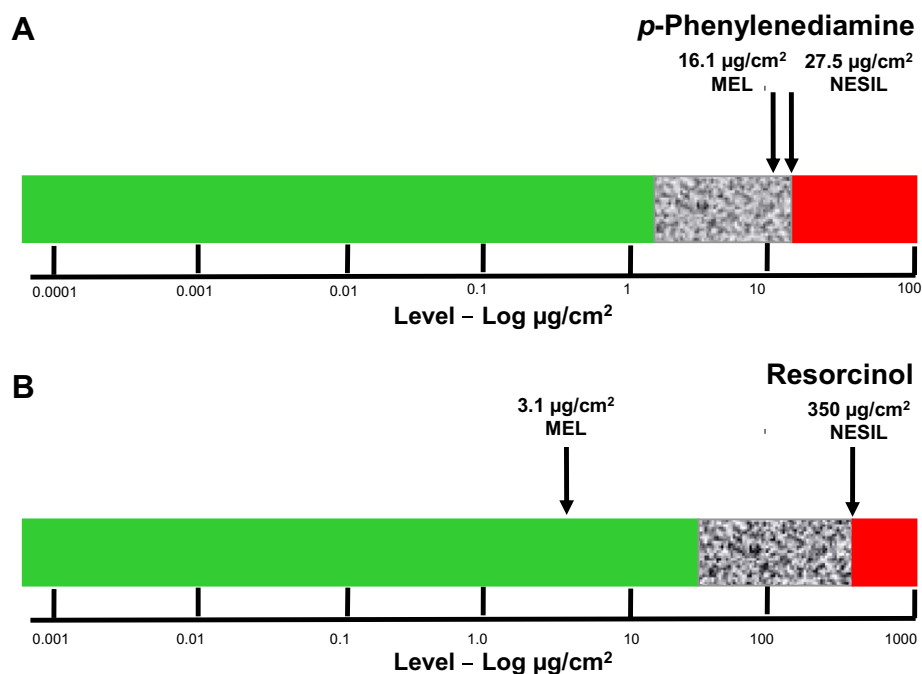


Fig. 1. Quantitative comparison of exposure doses for skin sensitization induction and hair dye product usage. Logarithmic comparison of the sensitization induction potency indicated as NESIL (No Expected Sensitization Induction Level) and the actual hair dye product exposure under use conditions indicated as MEL (Measured Exposure Level) at maximal allowed on-head use concentrations of (A) 2% *p*-phenylenediamine (PPD), and of (B) 1.25% resorcinol. Red bar: exposure considered to be associated with induction of skin sensitization, green bar: exposure not considered being associated with the induction of skin sensitization, grey shaded bar: default value of 10 for inter-individual variability (modified from Basketter et al., 2003). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

zation a no effect level can be established and potential skin sensitizing ingredients can be used safely as long as skin exposure to them is sufficiently lower than the threshold for the induction of contact allergy. This is important because chemicals such as hair dyes and preservatives that are selected to perform a particular function related to their chemical reactivity are also considered to be associated with a higher degree of probability for a skin sensitization potential because of their chemical reactivity (Basketter et al., 2008b). Consequently, it is necessary to ensure an appropriate balance between functional performance and the risk of skin sensitization through the use of hair dye products. The successful achievement of this aim depends on an accurate identification of their sensitization potency under hazard assessment conditions as well as on a detailed knowledge of the consumer exposure under in use conditions (Gerberick et al., 2001b; Griem et al., 2003; Basketter et al., 2003; Basketter et al., 2005).

For hair dyes, both, the induction potency as well as the product exposure conditions are very well characterized as the EU regulation requires both data types for their safety assessment. For this purpose industry provided LLNA data and/or available Guinea pig and human data for the skin sensitization potency assessment as well as dermal absorption data for the systemic exposure assessment under hair dye product use conditions (SCCS, 2010a) in accordance with the relevant OECD guidelines and the principles of Good Laboratory Practices.

Complete LLNA datasets for induction potency determination under hazard assessment conditions were available from scientific publications for PPD (Table 1a, Basketter et al., 2007a) and resorcinol (Table 1b) as well as from regulatory sources (SCCP, 2006b; SCCS, 2010c). As presented in the results section consideration of all relevant available data on PPD led to a WoE NESIL of 27.5 µg/cm² indicating a strong allergenic potency of PPD under hazard assessment conditions. This is in line with human induction data from HMT and HRIPT testing (Table 2a).

For resorcinol the WoE NESIL of 350 µg/cm² was derived from the LLNA data set with the lowest EC3 value (Table 1b) using the vehicle DMF. This is considered a conservative approach as data generated using AOO as vehicle indicate a moderate skin sensitizing potency of resorcinol with an EC3 value of 5.5% (equivalent to 1375 µg/cm²). This is between 1 and 2 orders of magnitude less potent than PPD (Basketter et al., 2007b).

The actual exposures to PPD and resorcinol were determined from skin absorption studies performed according to relevant use conditions of hair dyeing, using the maximum on-head concentration of the investigated hair dye ingredient in order to derive the MEL (see Table 3). The MEL is equivalent to the sum of the amounts of ingredient in the receptor fluid, the dermis and the epidermis including the SC and thus represents a conservative value, as the SC is generally not considered to contribute to biological effects involved in skin sensitization (SCCS, 2010a; SCCS, 2010b). Furthermore as the MEL encompasses all amounts found on and in the skin and in the systemic circulation comparison with applied doses in skin sensitization studies (animal studies or clinical tests) is considered scientifically appropriate.

From a toxicological risk assessment perspective, the NESIL and the MEL for PPD and resorcinol represent two very reliable experimental descriptors obtained from studies thoroughly designed for this purpose. Consequently, SAFs accounting for uncertainties regarding different matrix effects and patterns of product use were not specifically considered. This is based on the fact that experimental vehicle matrices used in the LLNA for hair dye ingredients (mainly AOO, DMSO, DMF, see Table 1) are known to exhibit penetration enhancing and irritating effects (Woodford and Barry, 1986; Lashmar et al., 1989; Tsai et al., 2001) compared to hair dye product formulations. Furthermore, it has been shown that exaggerated increases in frequency of use such as one daily application of an oxidative hair dye product on three consecutive days in the LLNA are associated with an increased immune response (Bonefeld et al., 2010). If the increase observed in the LLNA re-

sponse is relevant for a single application of oxidative hair dye product occurring every 4–6 weeks or is due to an effect of repeated application independent of the hair dye ingredients remains to be investigated. However, provided adequate experiments would demonstrate that a single application to a hair dye in combination with other components of the formulation may impact the NESIL, this may be addressed by a corresponding SAF. Effects related to the persistence of hair dye ingredients in the skin are unlikely since within a period of 4 weeks, chemicals remaining on and in the SC and epidermis will be removed by continuous outward proliferation, differentiation and desquamation processes (Houben et al., 2007). In addition, the described pattern of usage is relatively uniform across all available hair dye products, and can be considered a typical exposure scenario required to obtain the desired hair color result. This is supported by use instructions provided as leaflet with clear guidance how and under which conditions the hair dye product may be or should not be applied. Consequently, as concentration of the hair dye ingredient is considered the most relevant variable for human exposure, its chemical potency is the primary determinant of the induction of skin sensitization.

Therefore, the direct comparison of MEL and NESIL is considered to provide a sufficiently conservative estimate of the skin sensitization induction risk of the individual hair dye ingredient under use conditions. This direct comparison of the MEL and the NESIL represents the proof-of-concept based on the data for PPD or resorcinol and their known allergenicity potential in the general population and among hair colorant consumers. Provided toxicologically reliable data for the MEL and the NESIL are available, estimation of the safety level of a hair dye ingredient in a hair colorant requires consideration of the SAF to account for the inter-individual variability in the population as this cannot be mimicked in the experimental studies. The generally suggested default value of 10 to account for individual susceptibility differences for the induction of skin sensitization (Felter et al., 2002) is also considered adequate for hair dye ingredients. This default value, as it is applied to the NESIL represents the experimental no effect threshold for induction, i.e. the low end of the induction distribution curve relevant for those individuals that are very susceptible and not to an average value derived from all individuals. Furthermore the exposure defined by the MEL includes the entire amount on and in the skin and is considered to substantially account for variability in skin barrier integrity. Although the individual susceptibility differences for the induction of skin sensitization (such as age, sex, race, genetic factors and compromised skin) are toxicologically covered by the generally accepted default factor of 10, this is not the case for elicitation. Apart from these general susceptibility differences, elicitation reactions may also depend on differences in the induction dose (Hostynek and Maibach, 2004) for which the default value of 10 may not be sufficient.

For PPD the relation between NESIL ($27.5 \mu\text{g}/\text{cm}^2$) and MEL ($16.1 \mu\text{g}/\text{cm}^2$) is depicted in Fig. 1A showing that NESIL $\geq$ MEL, i.e. the same order of magnitude with the NESIL only slightly above the MEL. This relation reflects the current market observation indicating that (a) the majority of consumers do not acquire skin sensitization to PPD through hair coloring, while (b) a minority of individuals may acquire skin sensitization to PPD through the use of hair colorants that contain PPD up to its maximal allowed use concentration of 2%. Consequently, the MEL lies in the grey shaded zone in Fig. 1A, suggesting that inter-individual variability was not sufficiently addressed. This view is supported by published data on patch test experience in different patient and non patient populations as well as cosmetovigilance experience (Thyssen and White, 2008; Krasteva et al., 2009; Krasteva et al., 2010). Based on the concept that MEL $\gg$ NESIL is associated with an increased likelihood to induce skin sensitization, a strong linear association

between PPD containing temporary black henna tattoos and the severity of patch test and clinical reactions has been described (Ho et al., 2005; Krasteva et al., 2010) as temporary tattoos are known to contain PPD concentrations of up to 64% (Almeida et al., 2011; Brancaccio et al., 2002).

For resorcinol, there are more than 2 orders of magnitude difference between the NESIL of $350 \mu\text{g}/\text{cm}^2$ and the MEL of $3.1 \mu\text{g}/\text{cm}^2$, i.e. NESIL $\gg$ MEL (Fig. 1B) indicating no or negligible risk of induction of skin sensitization when used up to its maximal use concentration of 1.25% in hair dye products. Consequently, the MEL lies in the green zone in Fig. 1A, suggesting sufficient consideration of inter-individual variability. This is also confirmed by published data on patch test experience in different patient and non patient populations (Basketter et al., 2007b; Frosch et al., 1993; Wang et al., 2011). Based on the concept that MEL $\gg$ NESIL is associated with an increased likelihood to induce skin sensitization, it was found that 23/24 resorcinol patch-test positive patients had been sensitized by an exposure to a prescription drug for wart treatment containing 20% resorcinol (Barbaud et al., 2001).

In conclusion, comparison of the NESIL and the MEL for PPD and resorcinol provided a scientifically relevant proof-of-concept quantification of the risk of skin sensitization associated with hair dyeing by confirming the observation in the market place. Comparison of these robust and toxicologically relevant values is therefore considered an improvement versus a hazard-based classification. Upon consideration of inter-individual variability, this comparison is considered a meaningful refinement to estimate safe exposure levels for new hair dye ingredients. Subsequently, analysis of human observational data from market as well as clinical epidemiological surveillance systems will provide a basis to evaluate the effectiveness of estimated safe exposure levels for the induction of skin sensitization.

Conflict of interest statement

Carsten Goebel, Maya Krasteva, Harald Schlatter, Jacquemine Pungier, Rolf Fautz, Anne Fuchs, Werner Schuh, and Frank Gerberick 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, Jean-Francois Nicolas, Brunhilde Blömeke, Pieter Jan Coenraads, Axel Schnuch, James Taylor and Ian Kimber were invited as consultants. The authors alone are responsible for the content and writing of the paper.

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All authors of this paper participated as active members in a workshop that was held in March 2011. As a follow-up of this workshop, the manuscript was composed based on the individual contributions. The manuscript is the joint work of the authors.

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