National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

A quantitative approach to assess the risk of skin sensitization from hair dye ingredients

A case study using p-phenylenediamine (PPD)

RIVM Letter Report 050012001/2013
J. Ezendam | M. Park | J.G.W. Salverda-Nijhof

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Rapport in het kort

Methode om een veilige limiet voor allergie door haarverf vast te stellen
Het gebruik van haarverf is in principe veilig. Wel bevat dit product stoffen die bij sommige mensen allergische reacties kunnen veroorzaken, zoals jeuk, roodheid en zwellingen van de huid. Zo reageert een klein percentage (0,2 tot 1 procent) van de consumenten op de stof *p*-phenylenediamine (PPD) in permanente haarverf. Haarverf-allergie kan in sommige gevallen ernstig zijn. Het RIVM heeft daarom geprobeerd te bepalen bij welke concentratie het gebruik van PPD niet meer zal leiden tot allergische reacties bij consumenten. Uit het onderzoek bleek dat het niet mogelijk was om deze veilige limiet te berekenen aangezien gegevens over de mate waarin de consument per verfbeurt aan PPD wordt blootgesteld ontbreken.

De veiligheid van haarverf wordt gereguleerd in de Europese Cosmetica verordening. PPD is een belangrijk bestanddeel van haarverf om grijs haar goed te kunnen kleuren, maar is ook een erkend allergeen. PPD mag tot een maximum concentratie van 2 procent worden toegevoegd aan haarverf. Deze wettelijke limiet voorkomt echter niet dat sommige mensen een allergische reactie krijgen na gebruik van haarverf.

Om een veilige limiet te kunnen bepalen is het nodig om de concentratie waarbij de stof de allergie veroorzaakt, de *effectconcentratie*, te weten. Er zijn voldoende gegevens over PPD beschikbaar om deze concentratie te bepalen. Daarnaast is het belangrijk om te bepalen in welke mate een consument wordt blootgesteld aan PPD bij het gebruik van haarverf. Als de hoeveelheid waaraan de consument blootstaat hoger is dan de effectconcentratie, kan een allergie ontstaan. Er zijn echter onvoldoende gegevens beschikbaar om de consumentenblootstelling met voldoende zekerheid vast te stellen. Eén van de redenen is dat de verf na een tijdje wordt uitgewassen en het onbekend is hoeveel PPD er in deze periode wordt opgenomen

Abstract

Method to determine a safe limit for hair dye allergy
The use of hair dye is in principle safe. However, the product contains chemicals that can cause allergic reactions, such as itching, redness and swelling of the skin, in some people. A small percentage (0,2 to 1 percent) of consumers react to the chemical *p*-phenylenediamine (PPD) in permanent hair dye, and this hair dye allergy can sometimes be severe. The National Institute for Public Health and the Environment has therefore tried to determine the concentration at which PPD does not elicit allergic reactions in consumers. This research concluded that it was not possible to determine such a safe limit as there was insufficient data to determine the concentration at which a consumer is exposed during the hair dyeing process.

The safety of hair dye is regulated by the European Cosmetics Directive. PPD is an important ingredient in hair dye necessary to cover grey hair, but it is also a known allergen. The maximum concentration allowed for PPD is 2 percent. Although, this legal concentration limit does not prevent some people from developing an allergic reaction after using hair dye.

In order to determine a safe limit, the concentration at which the chemical induces allergy, the *effect concentration*, needs to be assessed. Sufficient data was available to determine this concentration for PPD. In addition, it is important to determine consumer exposure during the hair dying process. If consumer exposure is higher than the effect concentration, there is a risk for allergy. There was insufficient data to determine consumer exposure. One of the reasons being, that hair dye is washed away after a short exposure time and the uptake of PPD in this period is unknown.

Contents

Executive summary

An important adverse health effect that can be caused by the use of permanent oxidative hair dyes is allergic contact dermatitis, which is characterized by skin rash, edema, and itching. This disease is induced by hair dye ingredients with skin sensitizing properties, for example *p*-phenylenediamine (PPD). The safety of hair dyes is regulated by the Cosmetics Product Directive and some substances are banned, while for other substances concentrations limits are defined. It is known that these limits are not sufficiently low to protect all consumers from becoming sensitized. For instance for PPD, the concentration limit is 2%, but this limit does not protect all consumers, since new cases of PPD allergy still occur. To estimate whether and at which level a hair dye ingredient is safe, a quantitative approach is required. Such quantitative risk assessment (QRA) approaches for skin sensitization have been described but have not been applied to hair dye ingredients yet. The aim of this study was to evaluate whether a QRA approach could be used for hair dye ingredients as well. To this end, a case study with PPD was performed in which the QRA method was applied to evaluate the maximum allowed PPD-concentration limit of 2%. An attempt was made to use the QRA approach to estimate a safe level for PPD.

For the QRA in this case study the guidance document of the International Programme on Chemical Safety of the World Health Organization (IPCS/WHO) was used. The key steps of this approach are effect assessment, exposure assessment and risk characterization. Effect assessment consists of hazard identification and characterization. For characterizing the skin sensitizing potential of chemicals, the point of departure is the NESIL: the *No Expected Sensitization Induction Level.* This NESIL can be derived from human studies and/or from animal studies (Local Lymph Node Assay (LLNA)). The AEL (A*cceptable Exposure Level*) is determined by dividing the NESIL by the total product of SAFs (S*ensitization Assessment Factors*). SAFs are applied to take into account uncertainties in the extrapolation of the experimental conditions to the real life situation. Application of a SAF for inter-individual differences is standard in this approach. SAFs to cover for interspecies differences, product matrix effects and product use and time considerations are applied on a case by case basis. The exposure assessment aims at estimating the CEL: *Consumer Exposure Level.* In the final step of the QRA, the risk is characterized by a calculating a risk ratio by dividing the AEL with the CEL. A consumer product is considered to be unsafe when this risk ratio AEL/CEL is lower than one and safe when AEL/CEL is equal to or higher than one.

The first step in the case study was the effect assessment for which both human and LLNA studies were retrieved. After evaluation of the available human studies, it was decided not to use the human data for deriving the NESIL. Both studies were not suitable due to limited information on the exposure concentrations. Therefore, the NESIL for PPD was based on LLNA data. Data from 13 LLNA studies were analysed with dose response modelling to identify a benchmark dose level representing the EC3 value. The EC3 value is the effective concentration required to produce a 3-fold increase of lymphocyte proliferation and can be used as the NESIL. The analysis resulted in a benchmark dose (BMD) level with a very narrow confidence interval, indicating that the EC3 value was quite consistent across the 13 independent studies. The lower limit of the BMD of 0.07% was converted to a dose per unit area, resulting in a NESIL of 17.5 $\mu g/cm^2$. In this case study the standard SAF of 10 for inter-individual differences

and a SAF of 3 for interspecies differences were applied, resulting in a total SAF of 30. Other SAFs were not included, since it was assumed that the uncertainties associated with the product matrix and product use and time were sufficiently accounted for in the exposure assessment. For PPD this resulted in an AEL of 0.58 μg/cm^2.

The next step in this QRA was the exposure assessment. The dermal load of PPD representing the external exposure dose was first estimated using the ConsExpo tool. The dermal load was calculated for a hair dye containing 2% PPD, resulting in an external exposure dermal load of 3448 μg/cm^2. This value represents the total dose that is applied on the hair for infinite exposure time. This is not a realistic scenario for hair dyes, since the exposure under hair dyeing conditions is approximately 30 minutes. Hence, the dermal load is an overestimation of the real exposure and this value is assumed to reflect a worst case scenario exposure. To obtain a more realistic CEL, two other scenarios were included in this case study. The first scenario was based on information available from human exposure studies. These studies showed that the majority of PPD is washed off after the 30 minute exposure. Based on these studies it was assumed that on average 5.8% of the dermal load of PPD is available for skin absorption (200 μg/cm^2). In the second scenario, it was assumed that only 0.1% of the dermal load would be available for skin absorption (3.448 μg/cm^2). This percentage was arbitrarily chosen and was based on the assumption that the majority of hair dye will be present in or on the hairs and will therefore not come in contact with the skin.

As a final step, the risk ratio was calculated for the three scenarios. In all scenarios, this ratio was below one. Hence, according to these scenarios the use of PPD at the maximum allowed concentration of 2% is not safe. It should, however, be noted that especially with respect to the exposure assessment many uncertainties exist and assumptions on the use of hair dyes were made. Subsequently, using the three scenarios, the concentration of PPD that would be safe for consumers was estimated, by assuming that the AEL represents a safe level. According to this rough estimate, hair dyes containing 0.00034%, 0.0058% and 0.34% PPD would be safe in the different exposure scenarios.

This case study shows that the currently allowed concentration of 2% PPD in hair dyes is not safe for consumers, which is substantiated by the new cases of PPD allergy that occur in the general population. It should be noted that in the QRA approach many uncertainties were encountered and many assumptions had to be made. The most important uncertainties and knowledge gaps identified include which SAFs should be included, whether the NESIL represents an internal or an external dose and the lack of good exposure data for hair dye. It became evident that hair dye is a specific consumer product category for which more insight in the actual consumer exposure and parameters that affect the on-head exposure is needed. A lot of uncertainties surround the use of the QRA approach for the risk assessment of hair dye ingredients and more specifically for proposing a safe concentration limit for PPD.

1 Introduction

Permanent oxidative hair dyes are an important cause of allergic contact dermatitis both in consumers and in workers, such as hair dressers (Schnuch *et al.*, 2008; Thyssen *et al.*, 2008; Uter *et al.*, 2007). The Scientific Committee on Consumer Safety (SCCS) of the European Union (EU) (formerly called the Scientific Committee on Consumer Products (SCCP)) identified several oxidative hair dye ingredients as skin sensitizers. Notably, the majority of these were categorized as extreme or strong sensitizers (SCCP, 2007). One of those potent skin sensitizers is *p*-phenylenediamine (PPD), an important human sensitizer that is included in several diagnostic patch test series. In Europe, the prevalence of PPD positive patch test reactions in patients with allergic contact dermatitis ranges from 2-6%. The prevalence in the general population is estimated to be between 0.2 and 1% (Krasteva *et al.*, 2009; Schnuch *et al.*, 2012; Thyssen and White, 2008). The clinical symptoms elicited by hair dye ingredients can be very severe and sometimes require hospitalization (Nosbaum *et al.*, 2012; Sosted *et al.*, 2006). In consumers, PPD allergy is mainly caused by the use of permanent hair dyes (LaBerge *et al.*, 2011). Furthermore, black henna, which is the combination of red henna and PPD that is frequently used for temporary 'black henna tattoos', can induce or enhance PPD-sensitisation. Tattoos are an important cause of PPD-induced allergic reactions in children and adolescents (Almeida *et al.*, 2011). Once sensitized to PPD, patients may experience allergic contact dermatitis from the use of PPD-containing hair dyes. Due to cross-reactions, an allergic response to other hair dye ingredients, textile dyes, local anaesthetics and rubber chemicals may occur in patients sensitized to PPD (de Groot, 2013).

In Europe, the safety of cosmetic products is regulated by the Cosmetic Products Directive (76/768/EEC) which was replaced by the Cosmetic Products Regulation (EC No 1223/2009) in July 2013. The Regulation prohibits and regulates the use of specific ingredients in cosmetics. Under the current EU legislation, certain hair dye substances are now banned, whereas for others concentration limits have been set. For PPD, the maximum allowed concentration in hair dyes, after mixing, is 2% (calculated as free base). Besides these restrictions, the Regulation also requires manufactures to inform consumers about the presence of allergenic substances such as PPD in hair dyes and mention these substances in the list of ingredients on the product leaflets. Most manufacturers advise consumers to perform an 'allergy alert test' on a small area of the skin prior to hair dyeing. This test is intended as a tool to assess whether a consumer might respond to the hair dye with an allergic reaction. In its present form, however, this test has many limitations and its usefulness as a preventive measure is subject of debate (Ezendam and Salverda-Nijhof, 2011; Orton and Basketter, 2012; Thyssen *et al.*, 2012).

The maximum use concentration for PPD is based on safety files submitted by industry. The observation that new cases of PPD allergy still occur amongst consumers (Søsted *et al.*, 2013) suggests that this limit is not sufficiently low to protect consumers from becoming sensitized. Since it is known that the induction of dermal sensitization is a threshold based phenomenon, the general toxicological principles of quantitative risk assessment can be applied to derive a safe level. For skin sensitizers quantitative risk assessment (QRA) approaches were developed that aim at estimating safe exposure levels for consumers. The

Research Institute for Fragrance Materials (RIFM) of the International Fragrance Organization (IFRA) has developed a specific QRA approach for fragrances (Api *et al.*, 2008). Based on this approach, IFRA provides the fragrance industry with so-called IFRA Standards that are used to formulate allergenic fragrance ingredients in consumer products. More recently, the International Programme on Chemical Safety of the World Health Organization (WHO/IPCS) proposed an adaption of the RIFM/IFRA QRA method that is applicable to all skin sensitizers (WHO/IPCS, 2012). In general, these approaches combine hazard characterization with an exposure assessment for a specific consumer product to come to a quantification of the risk. These quantitative approaches have been subject of debate (SCCP, 2008; Ter Burg *et al.*, 2010) and are not yet used to set safe levels for regulatory purposes.

Recently, a simplified version of the QRA method was used to assess the safety of the hair dye ingredients PPD and resorcinol (Goebel *et al.*, 2012). Their approach was not completely similar to the QRA approach of fragrances. The major difference was that the QRA was performed for another product category, namely hair dyes. It was demonstrated that using this simplified QRA, hair dyes containing the maximum allowed concentration of resorcinol were safe, whereas those containing the maximum allowed concentration for PPD may not be safe.

Outline of this report

The aim of this report is to further evaluate the QRA method for a quantitative risk assessment of sensitizing hair dye substances. In Chapter 2 of this report the QRA methodology for skin sensitization, based on the guidance described by WHO/IPCS (2012), is briefly described. Then, this QRA method is applied to assess the sensitizing properties of PPD in hair dyes, as a case study (Chapter 3), using different exposure scenarios. In addition, a safe concentration limit for PPD in hair dyes is estimated. The results of the QRA of PPD are evaluated and uncertainties and knowledge gaps are discussed in Chapter 4.

2 Methodology of the QRA for skin sensitization

2.1 QRA methodology

The QRA approach will be briefly described in this chapter. A more detailed description can be found in WHO/IPCS (2012). The key steps of the QRA for skin sensitization are illustrated in Figure 1 and further explained in the text below.

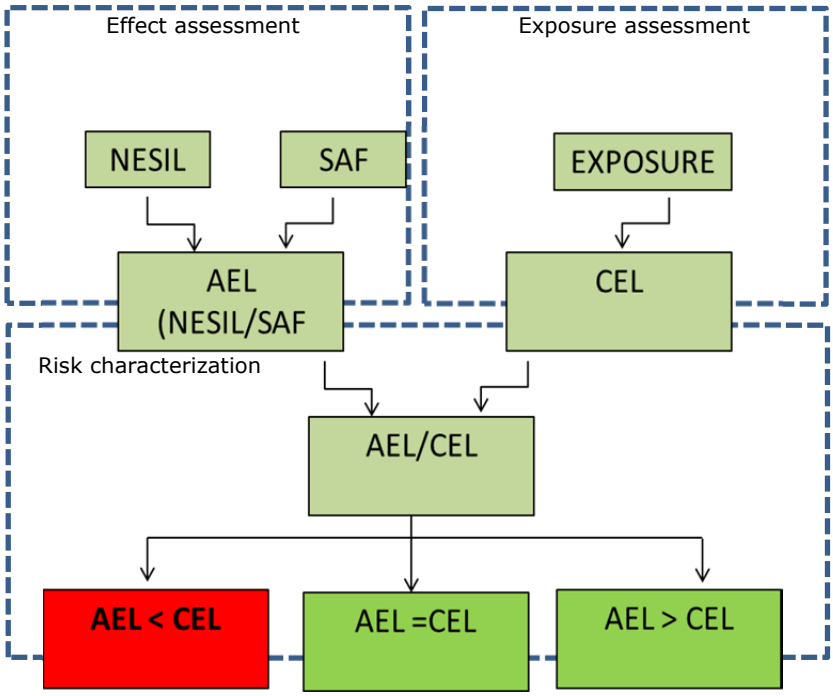

Figure 1: Key steps of the QRA method for skin sensitization
Abbreviations: NESIL= no expected sensitization induction level, SAFs = sensitization assessment factors, AEL = accepted exposure level, CEL = consumer exposure level. Adapted from (Loveless et al., 2010).

2.2 Effect assessment

A major factor in the risk assessment of sensitizers is the effect assessment, consisting of hazard identification and hazard characterization.

Derivation of the no expected sensitization induction level (NESIL)
According to the WHO/IPCS method the starting point for the effect assessment is the NESIL or *no expected sensitization induction level*. The NESIL is used as a quantitative point of departure (PoD) for risk assessment of skin sensitization induction and elicitation. WHO/IPCS recommends deriving a NESIL based on integration of all available data, both from human data, case reports and laboratory animal studies, using a weight of evidence approach (summarized in Table 1). Human skin sensitization studies include the Human Repeated Insult Patch Test (HRIPT) and the Human Maximization Test (HMT). Predictive sensitization testing in man, e.g. HRIPT, is considered unethical to conduct as stated by SCCP (2008) and in the outcome of a WHO-workshop (Van Loveren *et al.*, 2008). However, already published data can be used to derive the NESIL. The HRIPT is given precedence over the HMT. The reason for this is that in the

HMT the irritant sodium lauryl sulfate (SLS) is applied before exposure to the sensitizer of interest. For the assessment of skin sensitizing potency, this protocol is considered unsuitable, since the impact of the pretreatment with SLS on induction of sensitization is unknown. It is important to critically evaluate the study design of HRIPT, since in earlier times such studies aimed at assessing skin sensitization often used high concentrations. Such a design is not always suitable to derive a NOEL or LOEL. Table 1 summarizes the guidance provided by WHO/IPCS for the derivation of the PoD using human or animal data, including additional extrapolation factors that should be used to estimate the NOEL.

Table 1: Derivation of the Point of Departure (PoD) for risk assessment of skin sensitization[1]

Type of data	***Value used as PoD***	***LOEL to NOEL extrapolation***
Human		
HRIPT (or HMT)	NOEL ($\mu g/cm^2$ skin per day)	If a NOEL is lacking and results with sensitization rates below 50% are available the LOEL may be extrapolated by applying a factor of 3 to doses producing sensitization rates of 10-25% and a factor of 10 for sensitization rates of 25-50%
Animal		
LLNA	EC3 ($\mu g/cm^2$ skin per day)	None required

[1] Derived from the WHO/IPCS guidance (2012)

The LLNA (OECD Test Guideline 429) provides dose-response information that can be used to derive the EC3 value, which is the effective concentration required to produce a 3-fold increase of lymphocyte proliferation (Basketter and Scholes, 1992; Kimber *et al.*, 1991; Montelius *et al.*, 1994; Warbrick *et al.*, 1999; White *et al.*, 2006). This EC3 value correlates relatively well with the NOEL derived from human studies when the EC3 is expressed in $\mu g/cm^2$ skin (Gerberick and Robinson, 2000; Griem *et al.*, 2003; Schneider and Akkan, 2004). Therefore, the EC3 represents the NOEL and is used as the NESIL without applying additional NOEL extrapolation factors. Dependent on the available data, SAFs can be applied to account for inter-individual and interspecies differences (see chapter 2.2.2.).

Sensitization assessment factors (SAFs)
Sensitization assessment factors (SAFs) are applied to take into account uncertainties in the extrapolation of the experimental condition to the real life consumer situation. These factors are set between 1 and 10 and represent: (a) inter-individual variability; (b) interspecies differences when LLNA data are used in the absence of human data; (c) product matrix effects; (d) product use and time considerations. In short, the WHO/IPCS proposes to apply a factor of 10 for inter-individual variability, a factor that is commonly applied in risk assessment to account for possible variations in sensitivity between individuals. A SAF of 3 to account for interspecies variations is only applied if suitable human data are unavailable (Scheepmaker, 2006). Besides uncertainty factors that are routinely used in toxicological risk assessment, the use of additional factors has been proposed by IFRA/RIFM, to take into account special circumstances with regard to sensitization. The matrix factor, for example, is applied to take into account the differences between the experimental situation in which the NESIL is assessed and the real-life exposure to complex mixtures. This factor covers the uncertainty that these complex mixtures can contain ingredients that have

impact on the development of a sensitization reaction. The use and time factor is sometimes applied when the real-life scenario differs from the experimental situation in which the NESIL is established. It is intended to cover differences in skin site location, skin barrier integrity, occlusion and frequency of exposure. When these uncertainties are already covered in the exposure assessment (see section 2.3) a use and time factor should not be applied. WHO/IPCS recommends a case-by-case evaluation for both the matrix and the use and time factors.
To facilitate the definition of SAF values, IFRA/RIFM has identified a list of consumer product types with pre-set SAFs for uncertainties in inter-individual, matrix and use effects (Api *et al*., 2008). However, this list is specifically developed for cosmetic products containing fragrance ingredients and cannot readily be used in the effect assessment of hair dye products, when matrix and use effects are considered.

Determination of the accepted exposure level (AEL)
The accepted exposure level or AEL is determined by dividing the weight of evidence NESIL (expressed in $\mu g/cm^2$) by the product of the SAFs (described in the previous section). The AEL is used to define acceptable exposure levels to sensitizing agents. To this end, an exposure assessment is required.

2.3 Exposure assessment

Another essential element of the QRA approach is the exposure assessment. The purpose of this assessment is to understand how consumers are exposed in real life to sensitizing agents from the use of consumer products. An exposure assessment consists of a qualitative ('what products are used?') and a quantitative ('how much is used?') description of the contact of an individual with a chemical for a specific period of time (WHO/IPCS, 2009).

Determination of the consumer exposure level (CEL)
The guidance of WHO/IPCS on the QRA for skin sensitization (2012) does not provide any guidance on which exposure assessment method should be used. It describes some general methods that are available for this purpose. Additionally, Goebel et al. (2012) used an *ex vivo* skin absorption model for the assessment of PPD exposure. The applicability of these approaches to derive the CEL for PPD is discussed briefly.

- Category approach of IFRA/RIFM
  The QRA method described by IFRA/RIFM defines the CEL as a measure of consumer exposure under intended and foreseeable use conditions (Api et al., 2008). It takes into account the frequency and duration of use, consumer usage patterns (how do consumers use the product) and amount of product used per application/use. This category approach bases the exposure to a chemical in a specific product on the exposure estimate for the category to which that product belongs. This approach was specifically developed for products categories containing fragrances and does not include hair dye products. Therefore, this approach cannot be used in this case study for PPD.

- *Ex vivo* skin absorption models
  To assess skin absorption, *ex vivo* absorption models using excised human or pig skin, can be applied. The details of this test can be found in OECD guideline 428 (OECD, 2004). These models are designed to assess systemic

exposure, whereas for skin sensitization, the relevant exposure is the dose that is present in the epidermis during the application of the hair dye. It is complex to assess epidermal disposition using *ex vivo* absorption models (Basketter *et al.*, 2007). Progress is being made in this area by adapting the existing models to assess epidermal disposition (Davies *et al.*, 2011; Pendlington *et al.*, 2008). For PPD no epidermal disposition data were currently available.

- Estimation of external exposure using ConsExpo
  Another way of estimating exposure is using ConsExpo tool, version 4.1, developed at RIVM (www.consexpo.nl). The tool contains a set of models to assess external exposure to chemicals in consumer products. ConsExpo is accompanied by fact sheets on various groups of consumer products, which contain background information on the default models and parameter values selected for each product type, including hair dyes. These default values in the fact sheets are incorporated in the database of the ConsExpo software. ConsExpo has been selected as the method to estimate the CEL for PPD. An important consideration is that by selecting the default values from the database a worst case *external* exposure dose is obtained. The NESIL derived from LLNA data is also based on the *external* applied doses and it is assumed that the AEL therefore also represents an external dose and can be directly compared to this external CEL.

2.4 Risk characterization for skin sensitization

The risk for consumers is assessed by dividing the external AEL with the external CEL. When this ratio is below 1, the risk for consumers is considered to be unacceptable, whereas the product is considered to be safe when this ratio is equal to or higher than 1.
The uncertainties associated with the different steps of the QRA for hair dye ingredients will be discussed in more detail in the case study of PPD.

3 QRA approach for skin sensitization applied to PPD in hair dye

In this chapter, the QRA method was applied to assess the sensitizing properties of PPD in hair dyes, as a case study. To this end, different exposure scenarios are used. The key steps in the QRA for skin sensitization applied to PPD in hair dye are described below.

3.1 Effect assessment of PPD

3.1.1 Derivation of the NESIL for PPD

Human data
The NESIL is derived using a weight of evidence (WoE) approach taking into account human and animal data. As described in Chapter 2, retrospective use of human skin sensitization studies can be considered in such a WoE approach. For PPD, human data from sensitization tests with PPD were available from two studies, a HRIPT (Marzulli and Maibach, 1974) and a HMT study (Kligman, 1966).

In the available HMT study only a single dose of 71.4 µg/cm^2 was used together with a pretreatment with the irritant SLS. This treatment resulted in a sensitization rate of 21%; 5 out of 24 subjects were sensitized (Kligman, 1966). Because all subjects were pretreated with SLS and only a single dose was used, this study could not be used in the derivation of the NESIL.

In the available HRIPT study, three concentrations (0.01%, 0.1% and 1% PPD) were used. At the lowest dose, 7% of the subjects were sensitized. At the higher doses, these percentages increased to 11 and 53%, respectively (Marzulli and Maibach, 1974). As the dose metric for skin sensitization is µg/cm^2, the concentrations used in the HRIPT study have to be converted to the dose per unit skin area. The published study did not provide any information on the dose area that was used for the HRIPT study. Goebel et al. (2012) assumed that the concentrations in the HRIPT study were 10, 100 and 1000 µg/cm^2, respectively. The lowest dose in the HRIPT already induced sensitization, hence, 0.01% PPD (assumed to be equal to 10 µg PPD/cm^2) was considered to be the LOEL. The NOEL was then calculated by applying a factor of 3 (see Table 1), resulting in a dose of 3.33 µg/cm^2. No additional information was provided that substantiated their calculations. Due to these uncertainties it was decided not to use the information from the HRIPT study for de derivation of the NESIL, but to use available LLNA data.

Animal data (LLNA)
The EC3 is proposed to be a surrogate value for the human NESIL in risk assessment. Thirteen independent LLNA studies were used to derive the EC3 value for PPD (Kimber and Dearman, 1991; Montelius, *et al.*, 1994; Warbrick, *et al*., 1999). All studies used the same vehicle (4:1 olive oil/acetone (AOO)). For this case study, it was decided to deviate from the analysis method for LLNA data as described by the WHO/IPCS. The reason for this is that LLNA data from different experiments may be quite variable and this variation is not correctly reflected by taking an average of the EC3 values. To determine the EC3 and its

90% confidence interval, all published dose response data were analyzed simultaneously using the dose response modeling software PROAST (www.proast.nl). The PROAST software selects the optimal data fitting model from an exponential family of models using the likelihood-ratio criterion (Slob, 2002). The model fitting process also takes into account potential differences between studies and duplicate experiments by analyzing whether including 'experiment' as a potential covariate for the model parameters significantly improves the fit of the model to the data. Variation between animals within experimental groups could not be analysed since in all studies the lymph nodes were pooled for each experimental group.

Assuming that the data were still log normally distributed, the best fit to the data was achieved with the log-logistic model y = a * [c-(c-1)exp(-bx^d)], with experiment as a covariate for a. This yielded an average EC3 value of 0.08% and a relatively small 90% confidence interval of 0.07 to 0.11% PPD (Figure 2). This indicates that the LLNA provides a rather consistent EC3 value, despite some variation between experiments. The lower limit of the benchmark dose level is often used for risk assessment purposes and therefore the NESIL for PPD is 0.07%. To come to a dose per skin area, a conversion factor of 250 was applied (Griem, *et al.*, 2003), resulting in a NESIL of 17.5 µg/cm^2.

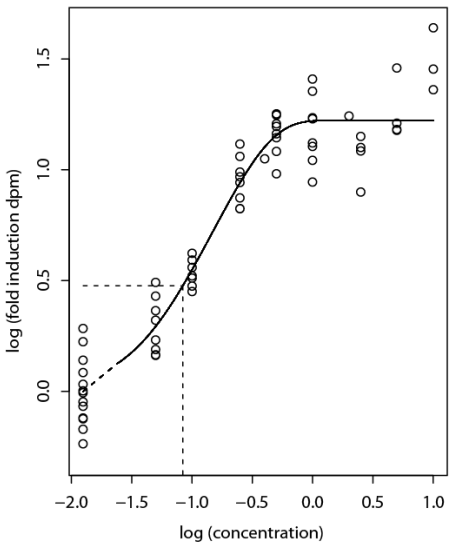

Figure 2: **Cellular proliferation as a function of PPD exposure in the LLNA based on data from 13 independent experiments**. Cellular proliferation was determined using incorporation of [^{3}H]TdR in the lymph nodes, which was measured by counting disintegrations per minute (dpm) per node for each experimental group. The best fit to the data was achieved with the log-logistic model y = a * [c-(c-1)exp(-bx^d)] with experiment as a covariate for parameter a. The dotted line represents a threefold induction compared to control, i.e. the EC3 value (%).

3.1.2 From NESIL to AEL: application of SAFs

For derivation of the AEL for PPD the following SAFs are considered relevant:

- A SAF for *interspecies differences* is included, since the NESIL for PPD is based on animal data and the human data were excluded. The guidance of

the WHO/IPCS proposed a SAF of 3 when the LLNA, in the absence of suitable human data, is used to derive an AEL. When a SAF of 3 is applied, a dose of 17.5/3 =5.83 µg/cm^2 is derived.

- The SAF for *inter-individual differences* was set at 10 as a default to account for variations in sensitivity of individuals within the human population.

- A SAF to cover uncertainties associated with the *product matrix* is not included. The reason for this is that uncertainties related to the product matrix will primarily impact the absorption of PPD and this should therefore be considered in the exposure assessment (see section 3.2) and not in the effect assessment. Additionally, the SAF for *product use and time* is not included. This SAF covers differences between experimental and real-life exposure. Again, these differences are taken into account in the exposure assessment for PPD, since in this assessment the duration of hair dye application and information from human absorption studies is taken into account.

Taken together, the total SAF in this case study is 30. The AEL is calculated by dividing the NESIL (17.5 µg/cm^2) with the total SAF (30), resulting in a dose of **0.58 µg/cm^2**.

The LLNA derived NESIL is in the same order of magnitude as the NOEL of 3.33 µg/cm2 estimated from the HRIPT (Kligman, 1966), making assumptions on the not reported surface area. Hence, the AEL resulting from human data would be in the same order of magnitude as the AEL from animal data.

3.2 Assessment of consumer exposure to PPD

This case study is restricted to the use of PPD permanent hair dyes by consumers. Occupational exposure is not considered. In the exposure assessment of PPD realistic hair dye conditions are included for a product that resulted in an on-head concentration of 2%.

3.2.1 Derivation of the consumer exposure level (CEL)

In this case study, the computational model ConsExpo version 4.1 was used to estimate the CEL for PPD. The instant application model is the default model in ConsExpo to assess exposure to chemicals in hair dye. This model assumes that all of the chemical in the product is directly applied to the skin and simply calculates the external exposure as the amount of product per surface area of skin (dermal load).
The dermal load is calculated as: $L_{derm} = (A_{prod} \times wf) / S_{exp}$.
where: L_{derm} = dermal load;
A_{prod} = amount of product applied to the skin;
wf = weight fraction of the chemical in the product;
S_{exp} = surface area of the exposed skin.

The calculation of the dermal load is based on the assumption that the maximum amount of hair dye product applied to the skin (A_{prod}) is 100 g (Bremmer *et al.*, 2006). The weight fraction (wf) of PPD in hair dye is 2%, which is the maximum allowed on-head use concentration in the EU. In line with the general fact sheet for ConsExpo, the surface area (S_{exp}) of the exposed skin is taken as half of the surface area of a head, which amounts to 580 cm^2

(Bremmer, et al., 2006). Based on ConsExpo modeling and using these default factors the dermal load is **3448 µg/cm^2**.

The dermal load expresses the *external* load of PPD when using hair dye assuming the scenario described above, i.e. instantaneous loading, without any information on duration. In real life, exposure to hair dyes is confined to approximately 30 minutes. The dermal load based on ConsExpo is therefore considered to be a gross overestimation of the actual exposure to PPD and as such a worst-case CEL.

3.2.2 Alternative exposure scenarios for hair dyes

To come to a CEL that will be more realistic for hair dye exposure two alternative exposure scenarios were included in this case study (summarized in Table 3).

The first alternative scenario is based on published information from human absorption studies to estimate which fraction of the applied dose will be available under realistic hair dyeing conditions, i.e. exposure to hair dye containing 2% PPD for 30 minutes. Two studies were found to be eligible for this and the details are described below and summarized in Table 3 (Hueber-Becker *et al.*, 2004; SCCP, 2006). In the study of Hueber-Becker *et al.* (2004) the absorption of 2% [^{14}C]-labeled PPD was assessed in eight male volunteers. In short, after the 30 minutes exposure, the hair was washed, dried, clipped and collected. Blood, urine and faeces were analyzed up to 120 hours after hair dyeing. The second human study was described in a recent SCCS opinion on PPD (SCCS, 2012). In this study absorption was investigated in 16 human volunteers (12 males, 4 females) who were exposed to a hair dye containing 2% [^{14}C] PPD. Following a 30 minute exposure, the hair dye was rinsed off, hair was dried and clipped and urine was collected for 48 hours. Table 2 summarizes the recovery of radiolabelled PPD in the different fractions that were sampled.

Table 2: Summary of human skin absorption studies

	Hueber-Becker, 2004	SCCS
	Average recovery (%)*	
Hair washes	81.7	61.5
Cut hair	13.0	31.7
Scalp washes	0.46	Unknown
Total non-absorbed	**95.16**	**93.2**
Recovery in urine, faeces	0.50	0.72
Percentage not-recovered	4.34	6.1
Percentage available for sensitization	**4.84**	**6.82**

* Recovery was expressed as the percentage of the total applied dose.

§ The percentage of the external dose available is the sum of the recovery in the scalp wash plus the percentage that was not recovered.

To estimate the percentage of PPD that is available to induce sensitization the following assumptions were made:

- PPD recovered in hair and scalp washes and cut hair is not available for skin absorption under realistic hair dye conditions.

- PPD recovered in urine and faeces has been absorbed and this percentage has been available in the epidermis and should be included in the percentage available for the induction of skin sensitization.
- The percentage PPD that is not recovered in this study is possibly absorbed and may still be present in the skin, although the authors stated that there also might be some PPD left in the hair not removed by clipping. As a conservative approach, it is assumed that the percentage of not-recovered PPD is available to induce skin sensitization.
- The percentage available for skin absorption is the sum of the recovery in urine and faeces and the percentage that is not recovered.

These studies in human volunteers show that under realistic hair dye conditions the majority of PPD is non-absorbed. The percentage that is available is 4.84% (Hueber-Becker et al, 2004) and 6.82% (SCCS, 2006). Based on these two studies the average percentage of the applied dose that is available for dermal absorption is 5.8%.

For the second alternative scenario it was assumed that only 0.1% of the total dermal load is available for dermal absorption. This percentage is arbitrarily chosen based on the assumption that a large fraction of the applied dose is on the hair and will not come in contact with the skin and therefore is not available to induce skin sensitization.

Table 3: Calculation of the CEL for PPD using three exposure scenarios

Scenarios	**Assumptions**	**PPD external dose ($\mu g/cm^2$)**
Classic CEL	The total dermal load calculated by ConsExpo is representative for the external dose. This is considered as a worst-case scenario	3448
$CEL_{5.8\%}$	Information from human studies was used to estimate the average percentage of applied PPD that is available for dermal absorption (5.8%)	200
$CEL_{0.1\%}$	A large proportion of PPD is applied to the hair and it is assumed that this is not available for skin absorption. In this scenario it is assumed that only 0.1% of the total load is available for dermal absorption	3.448

3.3 Risk characterization for PPD in hair dyes

In the QRA approach, the risk for consumers when exposed to PPD in hair dye is determined by comparing the AEL to the CEL (AEL/CEL). The risk is expressed as the AEL/CEL ratio and is calculated for the three exposure scenarios.
As shown in Table 4, all three exposure scenarios resulted in an AEL<CEL. Hence, based on this QRA approach and the assumptions made in this case study, it can be concluded that the maximum allowed PPD-concentration of 2% in hair dye is not safe. It should be noted that especially with respect to the exposure assessment uncertainties exist and assumptions were made. There is clearly a need for both better exposure data and more insight in the most relevant parameters that have an impact on the exposure assessment. This issue is further discussed in Chapter 4.

Table 4: Risk assessment of PPD in hair dye

Parameter	
NESIL (µg/cm^2)	17,5
Total SAFs	30
AEL = NESIL/SAF (µg/cm^2)	0.58
Risk characterization	
Classic CEL (µg/cm^2)	3448
Risk ratio AEL/CEL	**0.00017 (not safe)**
$CEL_{5,8\%}$ (µg/cm^2)	200
Risk ratio AEL/CEL	**0.0029 (not safe)**
$CEL_{0.1\%}$ (µg/cm^2)	3.448
Risk ratio AEL/CEL	**0.17 (not safe)**

3.4 Exercise in deriving a safe level for PPD in hair dyes

To continue this case study, the next step in the QRA approach is to re-evaluate the concentration of PPD in hair dye to estimate which concentration would be considered safe. For this exercise, the CEL has to decrease to a level equal to or lower than the AEL value, which means that the maximum acceptable CEL in this PPD case study is 0.58 µg/cm^2. Given the three exposure scenarios (100%, 5.8% and 0.1% of dose available for dermal absorption), the maximum dermal load and the related maximum allowable PPD concentrations were calculated using the ConsExpo model as described in section 3.2.1. Based on this model and the assumptions made with respect to the exposure, the maximum allowable PPD level ranges from 0.00034% to 0.34% (see Table 5).

Table 5: Exercise in estimating 'safe' levels of PPD in hair dye

Parameter	
AEL = NESIL/SAF (µg/cm^2)	0.58
Max CEL (µg/cm^2)	0.58
Risk characterization	
Dermal load assuming 100% available	**0.58 µg/cm^2**
'Safe PPD-level' = (0.58x580)/10^6	**0.00034%**
Dermal load assuming 5.8% available	**10 µg/cm^2**
'Safe PPD-level' = (10x580)/10^6	**0.0058%**
Dermal load assuming 0.1% available	**580 µg/cm^2**
'Safe PPD-level' = (580x580)/10^6	**0.34%**

4 Discussion and recommendations

In order to assess whether the QRA approach for skin sensitization could be applied to hair dyes, a case study with PPD was performed. Several uncertainties were encountered in this case study, especially with respect to the exposure assessment. With the current knowledge it is not possible to assess a realistic estimate of consumer exposure. An attempt was made to estimate the CEL using different exposure scenarios in which several assumptions were made. Importantly, in all scenarios, the currently allowed concentration limit of 2% for PPD in hair dyes is not safe for consumers. In fact, using the QRA approach for calculating a safe level for consumers and depending on the chosen exposure scenario, the maximum allowable PPD level would range from 0.00034% to 0.34%.

This case study revealed several uncertainties and data gaps that were either general aspects of the QRA approach for skin sensitizers, as discussed previously (SCCP, 2008; Ter Burg *et al.,* 2010), or specific issues when using this approach for PPD in hair dye products. The most important uncertainties are discussed briefly.

Effect assessment of PPD in hair dyes

- To come from a NESIL to an AEL, only two SAFs were applied, accounting for interspecies and inter-individual differences. There is an overall agreement to use a factor of ten for the inter-individual differences, although the SCCP (2008) commented that differences up to 100 were found in a human sensitization study. In a study by Kligman *et al.* (1966) it was shown that a concentration of 10% PPD sensitized all subjects, whereas a concentration of 0.1% only sensitized 21% of the subjects. Due to several uncertainties in the available human studies, it was decided to use available LLNA data and apply a SAF for interspecies and inter-individual differences.
- It was decided not to include the SAFs taken for matrix effects and use considerations in the effect assessment. The reason for this is that these uncertainties should be covered in the exposure assessment. It is still a matter of debate whether this is the correct approach, especially since differences exist between exposure in the LLNA and the actual consumer exposure, both in the matrix and the use of the product. These differences can affect the EC3 value as well. In an attempt to take these differences into account in the exposure assessment, we included a scenario based on human skin absorption data of studies performed under realistic hair dyeing conditions.
- For PPD a large number of LLNA studies were available and by applying dose-response modelling it was shown that these studies resulted in a very consistent EC3 value. This strengthens our confidence in using the EC3 value as a PoD for the QRA. Although we chose not to use the human studies for the AEL derivation, the AEL based on a human NOEL from the HRIPT (0.33 $\mu g/cm^2$; derived with assumptions on surface area and SAF of 10 for inter-individual differences) was in line with the AEL derived from the LLNA data (0.58 $\mu g/cm^2$).

NESIL based on LLNA EC3: an internal or external dose?

- One issue that was raised in the comparison of the NESIL and the CEL is whether the EC3 should be considered as an internal or an external dose. In this case study it is assumed that the LLNA EC3 is representative for an external dose and should therefore be compared to the external CEL. It is important to note that the exposure in a LLNA test differs from consumer exposure. The test substance is applied 3 times on the mouse ear skin, which is very thin. Furthermore, in most instances a high dose is used in a vehicle that will most probably enhance penetration. Hence, in the LLNA rapid skin absorption can be assumed and the doses applied might be representative for the internal dose as well. To conclude, there is a clear need for more insight in the exposure scenarios that are realistic in the experimental setting (LLNA) and under hair dye conditions in consumers.

Exposure assessment PPD in hair dyes

- One of the important limitations that was revealed in this case study was the lack of data on actual consumer exposure to PPD. For this reason there was a high level of uncertainty in the risk characterization, which is based on the comparison of the CEL with the AEL. With regard to hair dye products, the most important uncertainties were related to the duration of exposure and the site of exposure. In this case study, we used the instant application model in ConsExpo to estimate the CEL, which is a measure for the total dermal load. This is clearly an overestimation of the actual exposure to PPD, which is also shown in human studies in which real life exposures were done (Hueber-Becker, *et al.*, 2004; SCCS, 2012). These studies showed that the majority of PPD was not absorbed in this period. This illustrates that hair dye products are a specific category that cannot be compared to either rinse-off (short exposure) or leave-on products (infinite exposure).

- The site of application, being primarily the hair, also raised several uncertainties that impaired the exposure assessment. The factsheet of ConsExpo estimated the total load based on the area of the head, divided by two. It is unknown whether this area is representative for the actual skin area where absorption takes place. It is possible that the hair acts as a reservoir, since hair dye ingredients are designed to cross-link to the hair. The human skin absorption studies already demonstrate that a large proportion of the applied dose is present in or on the hair and is not absorbed during the 30 minute exposure. For this reason, the low skin availability scenario (0.1%) was included as well.

- Evidently, there is a clear need for better exposure data and more insight in the most relevant parameters that have an impact on the exposure assessment. Epidermal disposition is considered to be the most relevant metric for skin sensitization, since the induction of sensitization will take place in the epidermis (Basketter, *et al.*, 2007). Some progress is being made in this area and in the future models that can determine epidermal disposition can be of value in the QRA approach.

5 Conclusions

This case study shows that the currently allowed maximum concentration limit of 2% for PPD in hair dyes is not safe for consumers. This conclusion is in line with the paper by Goebel et al. (2012) who used a different QRA approach but also showed that exposure to 2% PPD is associated with induction of skin sensitization. The fact that PPD, regardless of the concentration limit of 2%, still leads to new cases of PPD allergy in the population, further substantiates our conclusion (Schnuch, *et al.*, 2012; Sosted *et al.*, 2013).

Using several assumptions, safe exposure levels were estimated using three exposure scenarios. However, due to the uncertainties in the QRA for hair dye ingredients it is currently not possible to decide which scenario is the most realistic. Hence, we are not able to propose a safe concentration limit for consumers in order to prevent new cases of PPD allergy. At this moment, industry (RIFM) is working on further refinement of the QRA method for the assessment of sensitizers in general. Depending on the developments in the area, the QRA for hair dye substances might be further developed, making it possible to derive safe product levels. It should be noted that the QRA method aims to protect consumers from becoming sensitized, but it is not suitable to protect already sensitized individuals. Also, occupational exposure is not addressed in the QRA and hair dressers should therefore be well educated in the possibilities of preventive measures.

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