EUROPEAN COMMISSION

Health & Consumer Protection
Directorate-General

Scientific Committee on Consumer Products

SCCP

OPINION ON

Intermediates and reaction products of oxidative hair dye ingredients formed during hair dyeing

Scientific Committees
on consumer products
on emerging and newly identified health risks
on health and environmental risks

The SCCP adopted this opinion at its 19th plenary of 21 January 2009

About the Scientific Committees
Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Products (SCCP), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCP
Questions concerning the safety of consumer products (non-food products intended for the consumer).
In particular, the Committee addresses questions related to the safety and allergenic properties of cosmetic products and ingredients with respect to their impact on consumer health, toys, textiles, clothing, personal care products, domestic products such as detergents and consumer services such as tattooing.

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1. BACKGROUND

Based on a mandate by the Commission, the Scientific Committee evaluated the scientific paper 'Use of permanent hair dyes and bladder cancer risk' by M. Gago-Dominguez et al (Int. J. Cancer: 91, 575-579 (2001)).

On 12 June 2001, the SCCNFP adopted during its 17th Plenary Meeting the first opinion (SCCNFP/0484/01) on the use of permanent hair dyes and bladder cancer risk with the following recommendations:

- *"the European Commission provides resources for the urgent review of the information;*
- *further epidemiological studies are performed to evaluate the possible association between bladder cancer and the use of permanent hair dyes in the EU;*
- *the European Commission takes further steps to control the use of hair dye chemicals since the potential risks of using this category of substances give cause for concern*".

In view of the safety concerns expressed in relation to the use of hair dyes, the Commission agreed in April 2003 together with Member States and the stakeholders on an overall strategy to regulate hair dye substances within the framework of the Cosmetics Directive. The strategy was published as an "Information note on the use of ingredients in permanent and non-permanent hair dye formulations (dye precursors and direct dyes)" on the DG ENTR website.

The main element of the strategy is a three step modulated approach requiring industry to submit, by certain deadlines, safety files on hair dye substances for a risk assessment by the Scientific Committee on Consumer Products (SCCP) according to the most recent safety requirements.

The hair dye strategy foresees to ban all permanent and non-permanent hair dyes for which industry has not submitted any safety files, and those, for which the SCCP has given a negative opinion.

The overall objective of this assessment process is to establish a positive list of hair dye substances, which are considered safe for human health and allowed for use by the cosmetics industry.

Epidemiological studies and the development in a possible causality between use of permanent hair dyes and bladder cancer have constantly been followed by the SCCNFP and SCCP. The following opinions have been adopted as follow up to the first opinion from 2001:

- SCCNFP opinion (SCCNFP/0797/04) concerning Use of Permanent Hair Dyes and Bladder Cancer. Updated 2004, adopted 23 April 2004.
- SCCP opinion (SCCP/0930/0) on Personal Use of Hair Dyes and Cancer Risk adopted 20 September 2005

An essential part of the hair dye strategy was the requirements for testing hair dye substances. This element has been addressed in the following opinions:

- SCCNFP Proposal (SCCNFP/0553/02) Assessment Strategies for Hair Dyes adopted 17 December 2002.
- SCCNFP Proposal (SCCNFP/0566/02) for A Strategy for Testing Hair Dye Cosmetic Ingredients for their Potential Mutagenicity/Genotoxicity adopted 4 June 2002.

- SCCP Opinion (SCCP/0971/06) on Updated Recommended Strategy for Testing Oxidative Hair Dye Substances for their Potential Mutagenicity/Genotoxicity adopted 28 March 2006.
- SCCP Opinion (SCCP/0959/05) on Review of the SCCNFP opinion on Hair Dye Strategy in the light of additional information adopted 20 June 2006.

The 3rd step of the modulated hair dye strategy and the last part of the hair dye strategy is to evaluate the reaction products actually formed when dyeing the hair. The current submission by COLIPA[1] submitted by December 2007 provides this final step 3 in form of a position paper of the industry for evaluation by the SCCP.

2 opinions concerning the reaction products have already been adopted by the SCCP:

- SCCP opinion (SCCP/0941/05) on Exposure to reactants and reaction products of oxidative hair dye formulations adopted 13 December 2005.
- SCCP opinion (SCCP/1004/06) on Update of the Annex to the Opinion on Exposure to reactants and reaction products of oxidative hair dye formulations adopted 20 June 2006.

2. TERMS OF REFERENCE

With the current submission from Industry, does the SCCP consider that further testing with regard to potential mutagenicity and carcinogenicity of intermediates and reaction products of oxidative hair dye products formed during the hair dyeing process, is needed?

[1] COLIPA - European Cosmetics Toiletry and Perfumery Association

3. OPINION

3.1. Introduction

In its opinion of December 2002 on "Assessment Strategies for Hair Dyes" SCCNFP/0553/02 (Ref. 1), the SCCNFP recommended an overall safety assessment strategy for hair dyes including the requirements for testing hair dye cosmetic ingredients for their potential genotoxicity and carcinogenicity. The main element of the strategy is a tiered, modulated approach requiring industry to submit by certain deadlines files on hair dyes to be evaluated by the SCCNFP. According to the hair dye strategy, dossiers for combinations of ingredients in permanent hair dyes should be submitted by industry at the latest December 2007 for the evaluation in Scientific Committee on Consumer Products (SCCP).

On 23 April 2004, SCCNFP evaluated industry's first submission "Ring Study on Reaction Products from Typical Combinations of Hair Colouring Ingredients" (SCCNFP/0808/04, Ref. 2). It was concluded that the analytical method developed for the determination of reactants and reaction products of oxidative hair dye formulations is based on sound chemistry and that it is validated. However, both qualitative and quantitative information on reactants and reaction products of various hair dye formulations, under use conditions, were required for the safety evaluation of these products. In January 2005, industry submitted a technical report addressing exposure to reaction products during hair dyeing process. This report provided additional information on reaction products of several combinations of oxidative hair dye ingredients. SCCP evaluated this submission and came to following conclusions (SCCP/0941/05, Ref. 3):

An analytical methodology based essentially on HPLC was developed, which allowed to follow oxidative hair dye coupling chemistry under conditions reflecting consumer usage. The methodology applied to 11 different combinations of hair dye precursors and couplers revealed:

- Expected reaction products based on the chemistry of oxidative coupling of precursors and couplers were obtained.
- No significant additional reactions or unexpected products were detected.
- The appearance of coupling products (dimers and trimers) can be detected and quantified.
- Levels of reactants (precursors and couplers) and reaction products (hair dye) can be determined in the formulation.
- Extraction of dyed hair allows the quantification of reactants in the dyed hair.
- The overall experimental recovery obtained from combined levels detected in the hair and in the formulation is close to 100%.
- Self-coupling products (such as Bandrowski´s Base) or transient intermediates were not detected in the hair dye formulation.
- In complex mixtures, the chemistry of the binary combination with the fastest kinetics will dominate.
- The concentration of reaction products in the formulations after 30 min varied from 0.05 % (*Combination A5+A44*) to 0.65% (*Combination A154+A27*).
- During the dyeing process, the consumer is exposed to the precursor(s), coupler(s) and expected reaction product(s).
- Although transient quinonediimine intermediates, essential for the formation of oxidative coupling of precursor and coupler in hair dye formulations, were not found in the present investigation, exposure of consumers to these molecules cannot be ruled out.
- The cream base used for the formulation of precursors and couplers contained only basic ingredients but that was not similar to marketed products, which may contain various other ingredients such as colorants. Thus, the influence of these other

ingredients on oxidative coupling of precursors and couplers, as well as formation of new molecules is not envisaged in this study.
- The SCCP understands that over one hundred different precursors and couplers are used in oxidative hair dye formulations in the EU. Studies, similar to those presented here, with all the most relevant combinations of precursors and couplers should be performed to obtain necessary information on consumer exposure.
- As the reaction products (hair dyes) of oxidative coupling of different precursor-coupler combinations (available to consumer) can be predicted, these should be synthesised. Their percutaneous absorption characteristics should be evaluated and in case of significant systemic exposure, further relevant toxicity studies are required.

Industry also submitted physico-chemical properties and *in vitro* dermal absorption studies of nine reaction products of some active ingredients of oxidative hair dyes. The safety evaluation of these performed by SCCP was published in 2006 (SCCP/1004/06, Ref. 4). It was concluded that in some cases significant amounts of oxidative hair dye reaction products become systemically available to the consumer. Studies, similar to those presented, should be extended to include additional indicative combinations of precursors and couplers. According to the updated strategy of hair dyes (genotoxicity, doc n° SCCP/0971/06, Ref. 5) further testing may be required.

The aspect of allergenicity (skin sensitisation from intermediates as well as from newly formed compounds) was not addressed in the opinion.

The present submission "Industry Position on Reaction Products in Permanent Oxidative Hair Colouring Products" (Ref. 6) includes studies in response to the above mentioned conclusions together with a compilation of all studies on reaction products, performed so far by the industry, *in-vitro* dermal absorption of some reaction products a review of epidemiological studies concerning oxidative hair dyes and cancer, and finally, a suggestion for risk assessment methodology for oxidative hair dyes.

3.2. Oxidative hair dyes: reaction products of combinations of precursors and couplers

The chemistry of oxidative hair dye formation has been described earlier (SCCP/0941/05, Ref. 3). An example of formation of coloured molecule of oxidative hair dye by a combination of a precursor and coupler is shown in Figure 1. The cosmetic industry has in the past 5 years performed several studies to acquire the knowledge concerning reaction products of precursors and couplers of oxidative hair dyes to which consumers are exposed.

These studies include:

- Development of a suitable methodology for the identification and determination of both intermediates and reaction products,
- Investigation of intermediates and reaction products formed by the combinations of several precursors and couplers of oxidative hair dyes in relevant matrices in the absence of hair or in the presence of hair, simulating the hair dyeing process,
- Synthesis of several relevant reaction products, and studies of physico-chemical properties and dermal absorption,
- Exposure assessment of some reaction products based on dermal absorption studies performed *in vitro*

These studies demonstrated that the combinations of precursors and couplers mainly produce dimers and trimers as reaction products and no self coupling products such as Bandrowski's base are formed in the presence of a coupler. The other components of current commercial hair dye formulations, including direct dyes (HC Yellow 2, and 2-Amino-6-chloro-4-nitrophenol) have not been found to affect the kinetics of reaction products formation. Furthermore, no additional reaction products, other than those that could be predicted, were found. A study performed with three precursors (p-toluenediamine, p-

aminophenol, and 1-hydroxyethyl-4,5-diaminopyrazole) and three couplers (2-methylresorcinol, 4-amino-2-hydroxytoluene and 2,4-diaminophenoxyethanol) in a hair dye formulation revealed that the reaction products of oxidative coupling were in agreement with the theoretical predictions based on the reaction kinetics, and that fastest coupling reactions dominate the chemistry in the formulation.

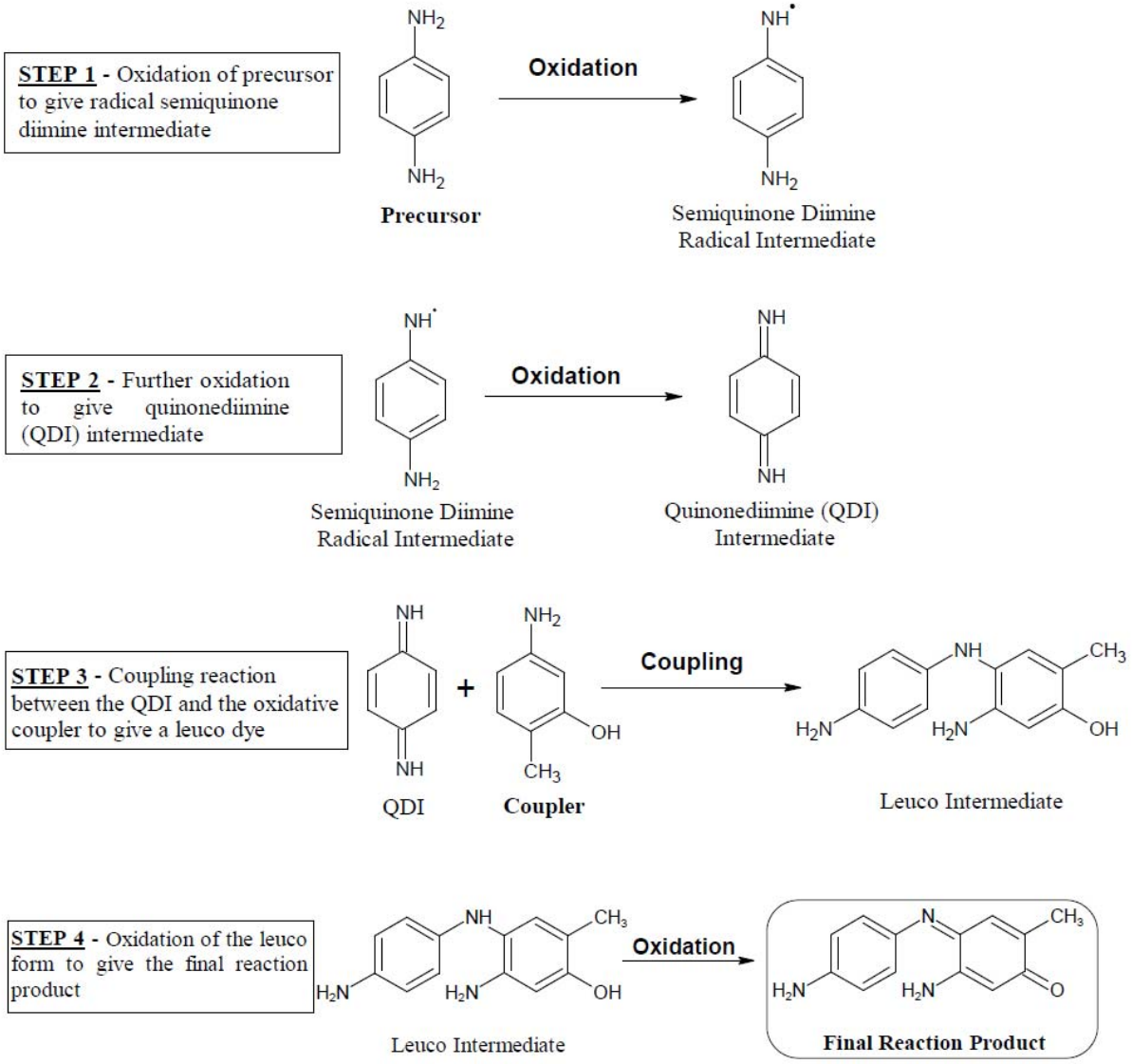


Figure 1: Proposed mechanism of oxidative hair dye formation

A review of the literature shows that for all of the kinetic studies carried out with oxidative precursors plus couplers and peroxide as the oxidant, the relative rates of each step is as shown in Figure 2 (from Ref. 6):

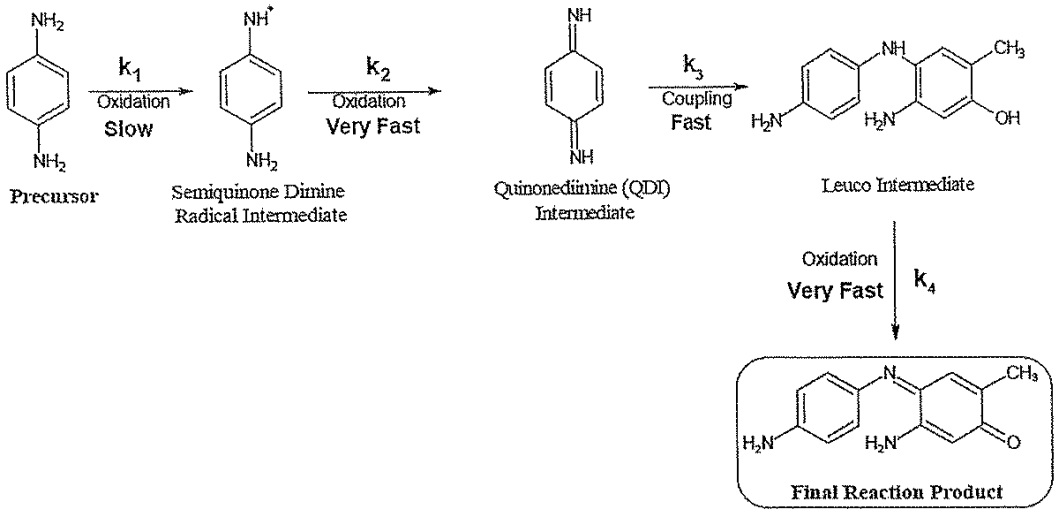


Figure 2: Relative reaction rates of various steps of oxidative hair dye formation

The rate of each step depends on the reaction conditions and especially the strength of the oxidant used. In the case of hair colouring products, the reaction proceeds in the presence of alkaline peroxide. In a series of studies on various oxidative coupling reactions under these conditions, Corbett (Ref. 7) has experimentally calculated the relative rates of each step and shown that:

$$k2 \text{ and } k4 > k3 >> k1$$

Corbett (Ref. 7) estimated that the rate of oxidation of p-phenylenediamine (PPD) to produce the QDI intermediate proceeds at a rate of approximately 0.002 min^{-1}. When this is compared to the rate with which QDI subsequently reacts with a coupler (Table 1), it is possible to calculate the steady state concentration of QDI. The theoretical maximum concentration of PPD-QDI in an oxidative hair dye formulation was calculated to be $23x10^{-9}$ molar or 2 ppb. The commercial hair colouring products contain antioxidants, which slow down the formation of QDI and further reduce the theoretical maximum concentration of QDI to below 2 ppb. Thus, < 2ppb PPD-QDI may be present in oxidative hair dye formulations during hair dyeing process, but the suspected build up of QDI in the hair dye formulation (SCCP/0941/05, Ref. 3) is theoretically not possible. A recent study, where mass balance of the precursor and reaction product was 100% at all times as the reaction proceeded, provided additional experimental evidence that there was no suspected build up of quinonediimine (QDI) intermediate. In addition, no QDI was detected in the oxidative hair dye formulations by a method employing a combination of analytical techniques (cyclic voltametry, electron spin resonance spectroscopy and UV/Vis spectroscopy). Finally, no leuco intermediates (Figure 2) of oxidative hair dyes were identified in the formulations simulating hair dyeing.

Table 1: Rate of formation of p-phenylenediamine (PPD)-QDI and rate of its coupling with some couplers (from Ref. 6)

Estimated Rate of QDI formation from alkaline peroxide = $0.002 min^{-1}$
Estimated Rate of QDI reaction with PPD = 28.7 L $mole^{-1}$ min^{-1}
Theoretical Max Concentration of QDI (no coupler) = 35×10^{-6} molar
Comparative Rate of Coupling with QDI

Coupler	Rate of Coupling with QDI	Relative Rate of Coupling with QDI
PPD	~ 30	1
Resorcinol	150,000	5000
1-Naphthol	74,000	2500
m-Aminophenol	55,000	1800

* Theoretical Max Concentration QDI (in presence of Coupler) = 23×10^{-9} molar or 2 ppb
* Theoretical Max Concentration QDI (in presence of Antioxidant) < 23×10^{-9} molar or 2 ppb

The chemical class of precursor and coupler determines the chemistry and the resulting reaction product structure. Table 2 summarises the combinations that have been studied and highlights:

- Examples of major precursor classes
- Examples of major coupler classes
- Highest tonnage precursors and couplers
- Most frequent combinations
- Combinations leading to dimeric reaction products
- Combinations leading to trimeric reaction products

The precursor and couplers studied so far can be grouped as follows.

Precursors

1. p-Phenylenediamines which include p-phenylenediamine (A007), p-toluenediamine (A005) and N,N-bis-hydroxyethyl-p-phenylenediamine (A050)
2. p-Aminophenols which include p-aminophenol (A016) and 4-amino-m-cresol (A074)
3. Heterocyclic diamines which include 1-hydroxyethyl-4,5-diaminopyrazole (A154) and 2,4,5,6-tetraaminopyrimidine (A053)

Couplers

1. Resorcinols which include resorcinol (A011) and 2-methylresorcinol (A044)
2. Blocked m-aminophenols leading to dimeric reaction products which include 4-amino-2-hydroxytoluene (A027) and unblocked m-aminophenols leading to trimeric reaction products which include m-aminophenol (A015)
3. m-Phenylenediamine derivatives which include 2,4-diaminophenoxyethanol (A042)
4. Pyridines which include 2-amino-3-hydroxypyridine (A132) and 2,6-Dihydroxy-3,4-dimethylpyridine (A099)
5. Naphthols which include 1-naphthol (A017)

The combinations of the seven precursors and ten couplers studied so far are described in Table 2. Thus, precursors and couplers with a variety of substituent such as hydroxy, amino, imino, carbonyl, hydroxyethyl, hydroxyethoxy and alkyl groups were included. The qualitative and quantitative information on the reaction products (dimers and trimers) formed is also compiled in Table 2. When precursors (approximately 62.5 µmol/g) and couplers (approximately 62.5 µmol/g) in an oxidative hair dye formulation reacted 30 min in

the presence of hair (ratio of hair dye formulation to hair 2:1, w/w), the reaction products formed were distributed in two compartments: within the hair and in the hair dye formulation surrounding hair including the hair surface. The free reaction products in the hair dye formulation remaining on the surface and outside hair are considered to be available for exposure of the skin (scalp). The concentrations of free reaction products of specific combinations of precursor and couplers reacted under the experimental conditions in the hair dye formulation outside the hair are given in Table 2. It should be noted that an additional compartment, the scalp, is also available for the distribution of hair dye formulation (and thus for the *in situ* dermal absorption of the reaction products formed) during hair dyeing by the consumer. The impact of this situation compared to *in vitro* hair dyeing using glass surface, as performed in the studies described here, has not been evaluated.

Table 2: Concentrations of reaction products formed from various combinations of precursors and couplers of oxidative hair dyes – amounts present were measured in the hair dye formulation on the surface and outside hair in experiments simulating hair dyeing

Oxidative combination	Reaction Product	Concentration (% w/w) in the formulation [a]
p-toluenediamine (A005) + 4-amino-2-hydroxytoluene (A027)	*Dimer A005-A027	0.26
p-toluenediamine (A005) + m-aminophenol (A015)	*Trimer A005-A015-A005	0.14
p-toluenediamine (A005) + resorcinol (A011)	*Trimer A005-A011-A005	0.03
p-toluenediamine (A005) + 2-methylresorcinol (A044)	*Trimer A005-A044-A005	0.02
p-toluenediamine (A005) + 2,4-diaminophenoxyethanol (A042)	*Dimer A005-A042	0.37
p-phenylenediamine (A007) + 4-amino-2-hydroxytoluene (A027)	*Dimer A007-A027	0.16
p-phenylenediamine (A007) + resorcinol (A011)	**Trimer A007-A011-A007	0.03
p-phenylenediamine (A007) + 1-naphthol (A017)	**Trimer A007-A017-A007	0.14
N,N-dihydroxyethyl-p-phenylenediamine (A050) + m-aminophenol (A015)	*Trimer A050-A015-A050	0.50
N,N-dihydroxyethyl-p-phenylenediamine (A050) + resorcinol (A011)	**Trimer A050-A011-A050	0.02
p-aminophenol (A016) + 4-amino-2-hydroxytoluene (A027)	*Dimer A016-A027	0.14
4-amino-m-cresol (A074) + 4-amino-2-hydroxytoluene (A027)	**Dimer A074-A027	0.08
1-hydroxyethyl-4,5-diaminopyrazole (A154) + 4-amino-2-hydroxytoluene(A027)	*Dimer A154-A027	0.65
1-hydroxyethyl-4,5-diaminopyrazole (A154) +m-aminophenol (A015)	*Dimer A154-A015 *Trimer A154-A015-A154	0.32 0.19
1-hydroxyethyl-4,5-diaminopyrazole (A154) +1-naphthol (A017)	**Dimer A154-A017	0.16
2,4,5,6-tetraaminopyrimidine (A053) + 2,6,dihydroxy3,4-dimethylpyridine (A099)	**Dimer A053-A099	0.08
4-amino-m-cresol (A074) + 1-acetoxy-2-methylnaphthalene (A153)	**Dimer A074-A153	0.20
1-hydroxyethyl-4,5-diaminopyrazole (A154) + 2,4-diaminophenoxyethanol (A042)	**Dimer A154-A042	0.33
1-hydroxyethyl-4,5-diaminopyrazole (A154) + resorcinol (A011)	**Trimer A154-A011-A154	0.06
2,4,5,6-tetraaminopyrimidine (A053) + 2-methylresorcinol (A044)	**Dimer A053-A044 **Trimer A53-A44-A53	0.04 0.02
2,4,5,6-tetraaminopyrimidine (A053) + 2,7-dihydroxynaphthalene (A019)	**Dimer A053-A019	0.03
p-toluenediamine (A005) +	**Trimer A005-A132-A005	0.16

2-amino-3-hydroxypyridine (A132)		
p-aminophenol (A016) + m-aminophenol (A015)	**Dimer A016-A015 **Trimer A016-A015-A016	0.05 0.06
p-aminophenol (A016) + 2,4-diaminophenoxyethanol (A042)	**Dimer A016-A042	0.14
p-aminophenol (A016) + 2-amino-3-hydroxypyridine (A132)	**Trimer A016-A132-A016	0.14
Precursor p-toluenediamine (A005) Couplers 4-amino-2-hydroxytoluene (A027)+ m-aminophenol (A015)	*Dimer A005-A027 *Trimer A005-A015-A005	0.21 0.10
Precursors: p-toluenediamine (A005) + p-aminophenol (A016) + 1-hydroxyethyl-4,5-diaminopyrazole (A154) Couplers 2-methylresorcinol (A044) + 4-amino-2-hydroxy-toluene (A027) + 2,4-diaminophenoxyethanol (A042)	Dimer A005-A027 Dimer A005-A042 Dimer A016-A027 Dimer A016-A042 Dimer A154-A027 Dimer A154-A042	[b] [b] [b] [b] [b] [b]

[a] in the hair dye formulation outside hair during simulating hair dyeing
[b] only qualitative analysis was performed
* studies which were submitted earlier and evaluated by SCCP
** studies not submitted for evaluation, only results were provided

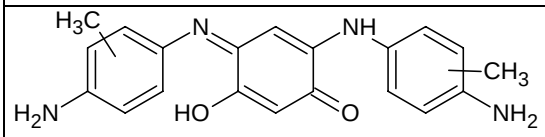
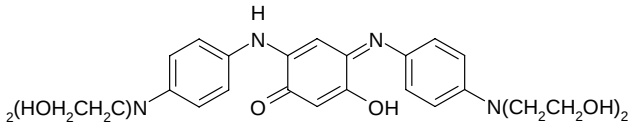
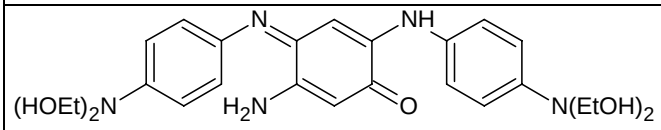
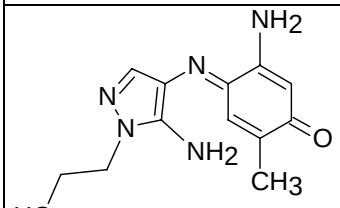
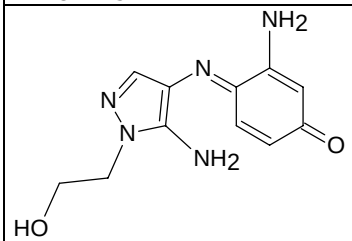
3.3. Exposure: Dermal absorption

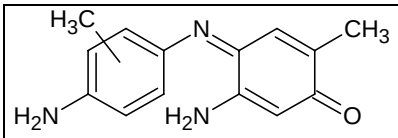
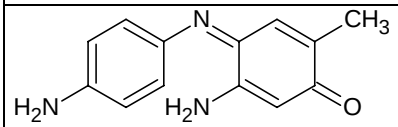
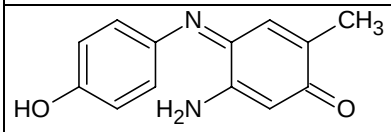
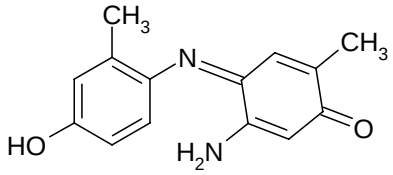
Industry has earlier submitted physico-chemical properties and *in vitro* dermal absorption studies of nine reaction products of oxidative hair dyes. The safety evaluation of these performed by SCCP was reported in 2006 (SCCP/1004/06, Ref. 4). In the present submission, additional studies have been submitted, with the intention of estimating the actual exposure during hair dyeing.

The earlier studies on *in vitro* dermal absorption of reaction products were performed under different conditions with respect to the presence or absence of hydrogen peroxide in the test material, concentration, duration of continuation of experiment after 30 min exposure, etc. (Table 3); and the rationale for the selected reaction products was not given. According to present submission, concentrations of reaction products used in the previous studies were much higher than the exposure concentrations. However, the test concentrations of hair dyes used in these tests were considered by SCCP as worst case.

Using human skin, the dermal absorption of 1% A005-A011-A005 in an oxidative hair dye formulation was investigated both in the presence and in the absence of hydrogen peroxide developer. The mean dermal penetration rates (epidermis + dermis + receptor fluid) were 0.076 µg/cm^2 and 0.092 µg/cm^2, respectively in the presence and absence of hydrogen peroxide. These results were taken as an indication that the presence of hydrogen peroxide developer may not have a significant effect on the dermal penetration of reaction products.

Table 3: Dermal absorption of oxidative hair dyes *in vitro*

Oxidative hair dye (reaction product of precursor and coupler in the presence of hydrogen peroxide)	Dermal absorption *in vitro* (µg/cm²) and experimental conditions Mean ± S.D. Range
 2-((4-amino-3-methylphenyl)amino)-4-((4-amino-3-methylphenyl)imino)-5-hydroxy-2,5-cyclohexadien-1-one Trimer A5-A11-A5	0.0920 ± 0.054 0.023 - 0.221 (a) 0.0767 ± 0.027 0.035 - 0.136 (b) Experimental conditions Concentration: 1% Skin: Human Duration after 30 min exposure: 48 h In the absence (a) and presence (b) of hydrogen peroxide
 5-Hydroxy-2-[[4-bis-(2-hydroxyethyl)amino]-phenyl]amino]-4-[[4-bis-(2-hydroxyethyl)amino]-phenyl]imino]- 2,5-cyclohexadien-1-one Trimer A50-A11-A50	0.032 ± 0.023 0.012 - 0.073 (b) Experimental conditions Concentration: 1% Skin: Human Duration after 30 min exposure: 48 h In the presence (b) of hydrogen peroxide
 5-Amino-2-[[4-[bis(2-hydroxyethyl)amino]phenyl] amino]-4-[[4-[bis(2-hydroxyethyl)amino]phenyl] imino]- 2,5-cyclohexadien-1-one Trimer A50-A15-A50	0.0789±0.046 0.015-0.182 (b) Experimental conditions Concentration: 1% Skin: Human Duration after 30 min exposure: 48 h In the presence (b) of hydrogen peroxide
 5-Amino-4-[(5-amino-1-(2-hydroxyethyl)-1H-pyrazol-4-yl)imino]-2-methyl-2,5-cyclohexadien-1-one Dimer A154-A27	0.009 ± 0.008 0.006 - 0.035 (a) Experimental conditions Concentration: 1% Skin: Pig Duration after 30 min exposure: 24h In the absence (a) of hydrogen peroxide
 3-Amino-4-((5-amino-1-(2-hydroxyethyl)-1H-pyrazol-4-yl)imino)-2,5-cyclohexadien-1-one Dimer A154-A15	0.006 ± 0.044 0.020 - 0.012 (a) Experimental conditions Concentration: 1% Skin: Pig Duration after 30 min exposure: 24h In the absence (a) of hydrogen peroxide

 5-Amino-4-((4-amino-2-methylphenyl)imino)-2-methyl-2,5-cyclohexadien-1-on, and 5-Amino-4-((4-amino-3-methylphenyl)imino)-2-methyl-2,5-cyclohexadien-1-one Dimer A5-A27	0.087 ± 0.051 0.025 -0.157 (a) Experimental conditions Concentration: 2% Skin: Pig Duration after 30 min exposure: 24h In the absence (a) of hydrogen peroxide
 5-Amino-4-((4-aminophenyl)imino)-2-methyl-2,5-cyclohexadien-1-one Dimer A7-A27	0.012 n.d. - 0.012 (b) Experimental conditions Concentration: 1% Skin: Human Duration after 30 min exposure: 24 h In the presence (b) of hydrogen peroxide
 5-Amino-4-((4-hydroxyphenyl)imino)-2-methyl-2,5-cyclohexadien-1-one Dimer A16-A27	0.150 ± 0.083 0.029 - 0.271(b) Experimental conditions Concentration: 1% Skin: Human Duration after 30 min exposure: 24 h In the presence (b) of hydrogen peroxide
 5-Amino-4-[(4-hydroxy-2-methylphenyl)imino]-2-methyl-2,5-cyclohexadien-1-one Dimer A74-A27	*0.02 ± 0.001* *Study was not submitted for evaluation, only resume' with incomplete details was available*

The new dermal absorption studies have been performed using three relevant concentrations of three reaction products which were followed over 72 hours after 30 min exposure (Table 4). For one combination (A5 + A27) only one concentration was investigated (Table 4). Dermal absorption studies with relevant concentration(s) of additional 13 other reaction products (Table 4) of oxidative hair dye ingredients are reported to be underway or planned. The criteria for selecting the reaction products in the new study were:

- Range of structure: Both dimeric and trimeric structures, and reaction products with a wide variety of substituents, such as hydroxy, amino, imino, carbonyl, hydroxyethyl, hydroxyethoxy and alkyl groups are included. Based on kinetics and chemistry, this set of 17 reaction products covers a large range of molecular weights and theoretically calculated water solubility
- Reaction products from the highest tonnage precursors and couplers.
- Frequently occurring reaction products formed during the use of commercial hair colouring product

The test materials in the new studies were typical oxidative hair dye formulations containing reaction products. The dermal absorption studies were performed in the presence or absence of hydrogen peroxide, depending on the stability of the reaction product. The test materials were applied to the skin mounted in diffusion cells at a nominal dose rate of 20

mg/cm^2. Following a skin contact period of 30 minutes, the surface of the skin was washed with mild detergent, simulating use conditions. Samples of receptor fluid were taken at regular time intervals over a 72 h period. At the end of the experiment, the surface of the skin was again washed and layers of the stratum corneum removed by tape stripping and the epidermis was separated from the dermis by heat separation.

Table 4: *In vitro* dermal penetration of the reaction products used in the new studies submitted

Reaction product	Exposure concentration during simulated hair dyeing % (w/w)	Test concentrations used in 72 h dermal penetration study % (w/w)	Dermal penetration ng/cm^2			Ref
			Receptor	Dermis	Epidermis	
			Mean ± SD			
A005-A011-A005	0.030	0.10 a	2.4±0.6	4.4±7.5	5.3±3.6	8
		0.30 a	4.7±1.9	6.5±5.1	36.0±27.9	
		1.0 a	11.7±4.1	33.0±32.9	32.6±33.8	
A050-A015-A050	0.250	0.25 b	0.8±0.5	1.4±0.9	15.8±17.6	9
		0.5 b	0.9±0.5	3.9±3.5	33.8±36.1	
		1.0 b	2.0±0.7	7.3±9.8	69.5±81.8	
A050-A011-A050	0.016	0.05 a	1.9±0.7	0.8±1.0	4.3±3.5	10
		0.1 a	2.7±2.0	0.9±0.6	4.6±6.3	
		1.0 a	5.3±2.6	2.9±2.6	40.1±32.7	
A005-A027	0.120	0.1 a	nd	7.1±10.7	6.4±8.9	11
Reaction products for the planned dermal absorption studies: A016 – A027, A005-A015-A005, A007-A017-A007, A007-A027, A154-A015, A007-A011-A007, A154-A017, A154-A027, A005-A042, A005-A044-A005, A053 – A099, A074 – A027, A007-A015-A007						

a: Test performed in the absence of hydrogen peroxide
b: Test performed in the presence of hydrogen peroxide
nd: not detected, limit of detection: 2.3 ng/ml

The design and performance of the new dermal absorption studies appears adequate, except for the following points:

- Not all studies were performed in the presence of hydrogen peroxide, and
- The HPLC method used for the quantification of the reaction product A005-A027 does not appear to be sensitive enough (detection limit ca. 2 ng/mL), because no A005-A027 was detected in the majority of the receptor fluid samples

3.4. Risk assessment

Based on the earlier submitted studies on *in vitro* dermal absorption of reaction products of precursors and couplers of oxidative hair dyes, SCCP concluded that in the worst case scenario of 1% hair dye exposure to 700 cm² (scalp), 0.14 – 3.16 µg/kg bw (person of 60 kg) of the hair dye will be absorbed (SCCP/1004/06, Ref. 4). Depending upon the toxicological profile of the hair dyes, some of these may be of concern. SCCP then recommended that similar studies should be performed which include more indicative combinations of precursors and couplers. According to the updated strategy of hair dyes (genotoxicity, SCCP/0971/06, Ref. 5) further testing may be required.

Since the earlier submitted *in vitro* dermal absorption studies had some shortcomings as described in chapter 3.3, industry performed further dermal absorption studies with 17 reaction products of various precursors and couplers of oxidative hair dyes. In the present dossier, however, *in vitro* dermal absorption studies of only four reaction products were submitted (Table 4). From the results, it is evident that absorption rates at lower concentrations of reaction products are lower compared to those at higher concentrations. In addition, from the kinetics of skin penetration over 72 h it can be deduced that not all of the test material that had penetrated into the skin is bioavailable. This should be considered when exposure is assessed (see also Ref. 12). Before any general conclusion on exposure of

hair dye reaction products can be drawn, the results of the additional dermal absorption studies have to be submitted and evaluated by the SCCP/SCCS.

Also, the proposal for using the threshold of toxicological concern (TTC) approach or the margin of exposure (MoE) concept for the risk assessment of oxidative hair dyes cannot be considered at this stage since reliable knowledge of exposure is a prerequisite. In addition, it has to be demonstrated that the available toxicity database of chemical compounds contains compounds similar to reaction products of oxidative hair dyes with regard to structural elements and complexity. Such a database should be established first. Furthermore, a detailed discussion of the structural elements of the hair dye reaction products with regard to structural alerts of genotoxicity and systemic toxicity is needed before the application of the TTC approach could be envisaged (Ref. 13). The application of the MoE approach in the case of reaction products is not relevant as it requires carcinogenicity data (Ref. 14).

3.5. Epidemiology

The International Agency for Research on Cancer (IARC) evaluated occupational exposures of hairdressers and barbers and personal user of hair colorants in addition to some hair dyes and cosmetic colorants in 1992 (IARC, 1993). It was concluded that there is limited evidence that occupation as a hairdresser or barber entails exposures that are carcinogenic and that there is inadequate evidence that personal use of hair colorants entails exposures that are carcinogenic. The conclusion with regard to occupational exposures was primarily based on an increased risk of bladder cancer. Eight individual hair dyes were evaluated. One of them showed sufficient evidence for carcinogenicity in animal experiments while limited evidence for carcinogenicity was found for four of the hair dyes evaluated. None of these hair dyes are at present permitted to be used in hair dye preparations in EU.

IARC repeated its evaluation in 2008 (IARC, in press). It was noted that many new epidemiological studies on cancer had been published since the last IARC assessment. IARC reconfirmed its conclusions from the evaluation in 1992.

Only studies concerning personal use of hair dye published after 1992 will be discussed in this chapter.

Although the evidence that personal use of hair dyes entails exposures that are carcinogenic is inadequate, some epidemiological studies on personal use of hair dyes raise concern especially in relation to bladder cancer and haematological malignancies.

Bladder cancer

Cohort Studies

A large prospective cancer mortality study (Cancer Prevention Study II, CPS II) conducted by the American Cancer Society has been published (Thun et al., 1994; Altekruse et al., 1999). This study collected data specific to personal use of permanent hair dyes in over 570,000 US women and found no association between permanent hair dye use and mortality from bladder cancer. Henley and Thun (2001) further extended the follow-up time for evaluation for this cohort. At this analysis there were a total of 336 bladder cancer deaths and the results continued to indicate no increased risk of bladder cancer associated with ever use of permanent hair dye.

Case-Control Studies

One case–control study from the Los Angeles area (Gago-Dominguez et al., 2001) suggested a possible association between personal use of permanent hair dyes and the risk of bladder cancer (OR= 1.4 (0.9-2.2)[2]), which was stronger for slow acetylators (OR= 2.9

[2] The numbers in brackets after the risk estimate represent the 95% confidence interval

(1.2-7.5))(Gago-Dominguez et al., 2003). This study was partly supported by a study from New Hampshire (Andrew et al., 2004). Among users of permanent hair dyes the risk of bladder cancer showed a non-significant increase among women in general (RR=1.5 (0.8-2.7)) and a significant increase among those that had started to use permanent hair dyes before the age of 37 (RR= 2.3 (1.1-4.6)) or started to use permanent hair dyes more than 31 years ago (RR= 2.6 (1.1-6.3)). On the other hand, a study from Texas (Lin et al., 2006) showed no significant increase in bladder cancer among users of permanent hair dyes either in the whole study or in any of the subgroups. Additionally, Kogevinas et al. (2006) did not find any increased risk of bladder cancer in a hospital based case-control study from Spain.

Lymphoma

Lymphoma is a broad term that includes a number of different cancers involving lymphocytic cells and the lymphatic system. There are two main groups of lymphomas - Hodgkin's disease and non-Hodgkin's lymphoma (NHL). All Hodgkin's disease lymphomas and most NHL are B-cell lymphomas.

Hodgkin's Disease

Cohort Studies

The American Cancer Society (CPS II) study collected data specific to personal use of permanent hair dyes and found no increased risk of death from Hodgkin's disease in over 570,000 US women included in the study (Thun et al., 1994). Another prospective cohort study on incidence of Hodgkin's disease was the Harvard Nurses' Health Study (Grodstein et al., 1994). This study included over 99,000 nurses and found no increased risk for Hodgkin's disease in relation to personal use of permanent hair dye.

Case-Control Studies

Several case–control studies of personal use of hair dyes have also been conducted, and these have not found any statistically significant association between Hodgkin's disease and either permanent or semi-permanent hair dye use (Miligi et al., 1999; Tavani et al., 2005; Benevente et al., 2005; de Sanjose et al., 2006). Only one study reported a statistically significant increased risk of Hodgkin's disease among female users of permanent hair dyes (Zahm et al., 1992).

Non-Hodgkin's Lymphoma (NHL)

NHL is a collective term applied to a group of many (depending on classification, more than forty different) lymphomas, all of which are clinically and biologically distinct subtypes. The classification or nomenclature given to these various NHL's has changed over the years. The current official classification system is known as the REAL/WHO system and is based on cell type, appearance, morphology, genetic features and immunologic phenotype.

There have been a large number of epidemiologic studies on personal use and occupational exposure to hair dye and NHL. Interpretation of the results is complicated by the changing nomenclature for the various NHL's over the years, gender differences in results in the various studies, combining data for all NHL subtypes rather than obtaining data and calculating risk for each specific subtype of NHL.

Cohort Studies

The American Cancer Society (CPS II) studied the association between mortality from NHL and permanent hair dye use (Thun et al., 1994; Altekruse et al., 1999). Overall, there were no associations between ever use of permanent hair dye or duration of use and NHL mortality. No increase in NHL was found in the Nurses' Health Study (Grodstein et al., 1994).

Case-Control Studies

Since 1992, 12 papers have been identified describing results from case-control studies of NHL and personal use of hair dye (Zahm et al., 1992; Linos et al., 1994; Holly et al., 1998; Miligi et al., 1999; Schroeder et al., 2002; Zhang et al., 2004; Chiu et al., 2004; Tavani et al., 2005; Benavente et al., 2005; de Sanjose et al., 2006; Chiu et al., 2007; Morton et al., 2007 (The results from Benevente et al., 2005 has later been included in the paper of de Sanjose et al., 2006)).

Two recent comprehensive review articles have been published on personal use of hair dyes and various cancers, including NHL. One study (Chiu et al., 2007) has not been considered in any of the review articles. Chiu et al (2007) analyzed tumour tissue samples obtained from a previous case-control study for the presence or absence of the chromosomal translocation 5(14;18). Hair dye use was not associated with either t(14;18)-positive or t(14;18)-negative subtype.

Takkouche et al. (2005) performed a meta-analysis for NHL based on 12 studies (Stavraky et al., 1981; Cantor et al., 1988; Zahm et al., 1992; Linos et al., 1994; Holly et al., 1998; Miligi et al., 1999; Schroeder et al., 2002; Zhang et al., 2004; Chiu et al., 2004; Tavani et al., 2005; Grodstein et al., 1994; and Altekruse et al., 1999) and showed that there was a statistically significant increased risk of NHL associated with ever users of hair dye (RR=1.23 (1.07-1.42)). The results for permanent dye use only (RR=1.13 (0.99-1.29)), based on 6 studies) and for intensive exposure (defined as more than 200 lifetime exposures to hair dye, RR=1.07 (0.90-1.28), based on 6 studies) did not show any statistically significant association. Takkouche et al. (2005) point out the fact that the restriction of the analysis to intensive exposure to hair dyes and to exclusive use of permanent hair dye did not strengthen the risk further, and they comment that this is "consistent with the absence of a causal effect".

The review of Zhang et al. (2008) included 4,461 NHL cases and 5,799 controls from the International Lymphoma Epidemiology Consortium (InterLymp). Three studies were from the USA, the Connecticut Women's NHL Study (CT) (Zhang et al., 2004), the National Cancer Institute (NCI)/Surveillance, Epidemiology) and End Results (SEER)' Multi-Center Case-Control Study (NCI/SEER), (Morton et al., 2007) and the Epidemiology of NHL Study from the University of California at San Francisco (UCSF) (Holly et al., 1998). The three U.S. studies collectively represent a total of six sites from the SEER program (Connecticut, San Francisco-Oakland, Iowa, Detroit, Seattle-Puget Sound, and Los Angeles). The case-control study from Europe (the EpiLymph International Case-Control Study of Lymphomas) (de Sanjose et al., 2006) represents geographic sites from six countries (Germany, Italy, France, Ireland, Czech Republic, and Spain). Each study collected detailed information on hair dye use (including duration of use, total number of applications, year of use, and type and colour of hair-dye used) and included histologically confirmed incident NHL cases.

Among women, 75% of the cases and 70% of the controls reported ever having used hair dyes. An increased risk of NHL was observed among women who started using hair dyes before 1980 compared with non-users (OR=1.3 (1.1-1.4)). Stratification by NHL subtype, hair dye use was associated with an increased risk of follicular lymphoma (FL) and chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL) but not other NHL subtypes. The increased risk of FL (OR=1.4 (l .l-1.9)) and CLL/SLL (OR=1.5 (1.1-2.0)) associated with hair dye use was mainly observed among women who started use before 1980 with a significant trend in risk with duration of use (P=<0.01, 0.02 respectively). For women who began using the products in 1980 or after, an increased risk of FL was limited to users of dark-coloured hair dyes (0R=1.5 (1.l-2.0)) with ORs of 1.5(l.1-2.1) for permanent dark-coloured hair dyes and 1.7(l.1-2.4) for non-permanent dark-coloured hair dyes. Results from the analyses showed that risk of CLL/SLL was increased mainly for European women who used hair dyes, not US women (de Sanjose et al., 2006, OR= 1.6 (1.1-2.5), Morton et al., 2007 OR=1.1(0.6-2.24), Holly et al., 1998 OR=0.8 (0.4-1.4), Zhang et al., 2004 OR=1.3(0.7-2.4)) whereas the association with follicular lymphoma was seen in both geographic regions. Although the duration and frequency of hair-dye use in the control

group were similar between European women and US women, duration of use among European women with CLL/SLL was greater than that for US women (p values ranged from 0.006 to 0.06 for various hair-dye products). Other factors, such as differences in hair-dye formulations, classification of rare NHL subtypes, or chance variations, also may have contributed to the observed difference in risk pattern between the European and US populations. Among men, approximately 10% of cases and 10% controls had ever used hair dyes. Risk of NHL was not associated with hair dye use before or after 1980 among men.

It is concluded that the results indicate that personal hair dye use may play a role in the risk of FL and CLL/SLL in women who started use before 1980 and that an increase in risk of FL in women starting use in 1980 or after cannot be excluded.

Leukaemia
In the SCCP Opinion (SCCP/093/05) it was concluded that "some studies indicate excess risk for acute leukaemia and chronic lymphoid leukaemia (CLL) for users of hair dye". As CLL is covered in the section on NHL, no discussion CCL is included below.

Cohort studies
Grodstein et al. (1994) using the Nurses Health Study women aged 30-55 years in 1976 followed to 1990), reported no increase in the risk leukaemia after use of permanent hair dyes.

Altekruse et al. (1999) reported from the American Cancer Society cohort study (CPSII) some indication for a possible increased risk of death from all leukaemia among women using permanent hair dyes (RR=1.1 (0.9-1.3)). The risk increased with duration of use: 1 – 9 years RR=0.9 (0.7-1.2), 10 – 19 years RR=1.2 (0.9-1.5), 20+ years RR=1.3(1.0-1.7), p-value for trend 0.04.

Case control studies
Mele et al. (1994) using patients from three hospitals in Italy found no statistically significant increased risks for acute myeloid leukaemia, acute lymphocytic leukaemia or chronic myeloid leukaemia.

Elevated risks for the dysmyelopoietic syndrome were also found in two studies from Japan with relative risks among women of 2.50 (0.97-6.41) (Ido et al., 1996) and 2.88 (1.38-6.01) (Nagata et al., 1999), respectively. In the study by Nagata and co-workers the RR=1.99 (1.17-3.38) for both sexes together. The risk increased with duration of use (10+ years RR=4.10 (1.64-10.23) and total frequency of use (70+ RR=3.08 (1.22-7.75)). No increase was found in a small group of men (OR=1.23 (0.53-2.88)). It is uncertain whether or not there is an overlap between the cases from the two Japanese studies. Personal communication to the author was not answered.

Miligi et al. (1999) found no increase among hair dye use and leukaemia. The risk was, however, increased among women using dark permanent products (2.0 (1.1-3.8)).

Rauscher et al. (2004) performed a population-based case-control study of acute leukaemia in 1986–1989 in USA and Canada. There was a modest positive association for ever use of hair dyes (OR= 1.3 (1.0-1.8)). The increase was stronger among those only using permanent dye (OR=1.6 (1.1-2.4)). The risk increased for long duration (15+ years) of use (OR=1.9 (1.1- 3.6)). The greatest odds ratio was for 15 or more years of using hair dyes and six or more times per year (OR=2.4 (1.0-5.8)). First use before 1970 represented a higher risk (OR=1.7 (1.0-3.0)) than first used after 1979 (OR=1.2 (0.51-2.9)). When stratified by leukaemia subtype, ever use of permanent hair dyes was associated with an OR of 1.6 (1.1- 2.5) for myelocytic leukaemia, and the trends in risk with duration and frequency were similar to the trends observed for all leukaemia subtypes combined. For lymphoblastic leukaemia, the OR for ever use of permanent dyes was 2.0 (0.9- 4.6). There

was a suggestion of a dose response for both duration and frequency, with the OR reaching 4.6 (1.5-14) for 15 or more years of use and the OR reaching 3.8 (1.2-12) for six or more applications per year. The authors conclude that long duration of permanent dye use may have a larger impact on the risk of adult acute leukaemia and other haematopoietic cancers than prior epidemiological data suggest.

3.6. Discussion

Chemistry

The methodology described earlier on the investigation of reaction products of precursors and couplers of oxidative hair dyes simulating the hair dyeing process, has been applied for the qualitative and quantitative analysis of 28 reaction products formed by 25 different combinations of seven precursors and ten couplers. In addition, one experiment was performed where only a qualitative analysis was done on the products formed by the reaction of more than one precursor and/or coupler in an oxidative hair dye formulation. The precursors and couplers used represented the highest tonnage precursors and couplers with a variety of substituents, such as hydroxy, amino, imino, carbonyl, hydroxyethyl, hydroxyethoxy and alkyl groups. The reaction products formed were of both dimeric and trimeric structures of a large range of molecular weight and water solubility. It was demonstrated that the other components of commercial hair dye formulations, including two direct dyes, did not affect the kinetics of reaction products formation; and no build up of any intermediate was observed. It was also revealed that the reaction products of oxidative coupling were in agreement with the theoretical predictions based on the reaction kinetics, and that the fastest coupling reactions dominate the chemistry in hair dye formulations.

The studies concerning reaction products of 11 combinations of precursors and couplers have been submitted earlier and they have been evaluated by SCCP. In the present submission, results of qualitative and quantitative analysis of the reaction products of 15 new combinations of precursors and couplers (excluding the qualitative analysis of reaction products of a combination of 3 precursors and 3 couplers) are described, but no documentation is provided. Thus, the studies concerning these have not been evaluated. According to the cosmetic industry, the concentrations of precursors and couplers used in these studies were the typical concentrations in the marketed products (approximately 62.5 μ mol/g in an oxidative hair dye formulation after mixing with hydrogen peroxide). Thus, the concentrations of the specific reaction products in the hair dye formulation to which the consumer is exposed, under conditions simulating hair dyeing, are 0.02 – 0.65% (Table 2). It should, however, be noted that the worst case scenario of the concentration of some active ingredients, for example p-phenylenediamine (A007) and toludine-2,5-diamine (A005) in hair dye formulations after mixing with hydrogen peroxide, can be >165 μmol/g according to the maximum allowed concentrations of these ingredients. To estimate relevant exposure concentrations of reaction products, the selected concentrations of the active ingredients, precursors and couplers, in the reaction mixture should represent the worst case scenario.

Although studies on the reaction products of precursors and couplers with several substituents have been performed, these do not include precursor/couplers with carboxyl-, nitro- and chloro- substituents. The reaction products of these may have significant different physico-chemical properties compared to those studied so far. Some examples of such precursors/couplers of oxidative hair dyes are:

A8: 2-Chloro-p-phenylenediamine (CAS No.615-66-7)
A94: 5-Amino-6-chloro-o-cresol (CAS No. 84540-50-1)
A117: 5-Amino-4-chloro-o-cresol HCl (CAS No. 110102-85-7)
B24: 4-Nitro-o-phenylenediamine (CAS No.99-56-9)
B28: Picramic acid (CAS No. 96-91-3)
B34: N,N'-bis(Hydroxyethyl)-2-nitro-p-phenylenediamine (CAS No. 84041-77-0)
B51: 4-Amino-3-nitrophenol (CAS No. 610-81-1)

Exposure/Dermal Absorption
Previously submitted *in vitro* dermal absorption studies with nine reaction products of precursors and couplers of oxidative hair dyes were performed using higher concentrations compared to exposure scenarios for hair dyeing, according to the applicant. Moreover, some parameters of the studies varied significantly from each other, for example the duration of continuation of experiment after 30 min exposure (Table 2). In the present submission, properly designed studies on three reaction products of oxidative hair dyes are included, and similar studies on 13 reaction products are reported to be either underway or planned. In addition, a dermal absorption study of a dimer (A 005–A027) was provided in the present submission, but only at one concentration. The performance of the new dermal absorption studies appears adequate, except that not all studies were performed in the presence of hydrogen peroxide, and the HPLC method used for the determination of A005-A027 appeared not to be sensitive enough.

The dermal absorption studies of the reaction products of oxidative hair dye ingredients should have been performed in the presence of hydrogen peroxide to mimic the consumer exposure scenario. It is mentioned that the test materials in the new studies were typical oxidative hair dye formulations containing reaction products; and these included either peroxide or placebo developer, depending on the stability of each reaction product. However, the new *in vitro* dermal absorption study of A005-A11-A005 was performed in the absence of hydrogen peroxide, although this molecule was stable in presence of peroxide developer: in one experiment in the previous submission, it was shown that the mean dermal absorption rates of A005-A11-A005 in the presence and absence of hydrogen peroxide were comparable. The degradation products of the hair dye reaction products which were not stable in the presence of hydrogen peroxide, are not described.

As the HPLC method used for the quantification of A005-A027 was not able to detect this molecule in several samples of receptor fluid, this method is not considered to be sensitive enough for the analysis of low levels of reaction products of oxidative hair dye ingredients. Use of radioactive test material and scintillation counting for determination should be the method of choice to study *in vitro* dermal absorption of reaction products of oxidative hair dyes in such cases.

SCCP will wait for the results of the studies which are reported to be underway or planned before drawing any conclusion on the basis of studies on only four reaction products. It is, however, recommended that *in vitro* dermal absorption studies should preferably be performed in the presence of hydrogen peroxide. Furthermore, reaction products with chloro- and nitro- substituent should also be included in the planned studies.

The instructions for use of commercial oxidative hair dye formulations also frequently recommend that the formulation should be applied on the hair for up to 45 min to achieve some specific shades/strong colours. In such cases, the exposure assessment should be performed on the basis of dermal absorption rate derived from an *in vitro* study, where skin membrane is exposed to test formulation for 45 min.

It is common that the marketed oxidative hair dye products contain more than 2 precursors and/couplers, and may contain even more than 5 precursors/couplers. Thus, the consumer during hair dyeing will be exposed to several reaction products simultaneously. In addition, the consumer will also be exposed to unreacted precursor/coupler at the same time. These situations should also be considered for risk assessment of oxidative hair dyes.

Epidemiology
Although the epidemiological evidence that personal use of hair dyes entails exposures that are carcinogenic is inadequate, some epidemiological studies on personal use of hair dyes may raise concern.

Some concern for bladder cancer has been raised on the basis of American studies. However, no increased risk has been found in a European study.

Some concern has been raised in relation to haematological malignancies. An increased risk of non-Hodgkin's lymphoma (NHL) was observed among women who started using hair dyes before 1980 compared with non-users. Stratification by NHL subtype, hair dye use was associated with an increased risk of follicular lymphoma (FL) and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) but not other NHL subtypes. The risk of CLL/SLL was increased mainly for European women who used hair dyes and not in US women. The results indicate that personal hair dye use may play a role in the risk of FL and CLL/SLL in women who started use before 1980 and that an increase in risk of FL in women starting use in 1980 or after cannot be excluded. Risk of NHL was not associated with hair dye use among men.

In a population-based case-control study of acute leukaemia in USA and Canada a modest positive association for ever use of hair dyes was observed. The increase was stronger among those only using permanent dye and the risk increased with number of years of hair dye use and number of use per year. First use before 1970 represented a higher risk than first used after 1979.

In the period from 1980, a number of colorants have been banned in EU as well as in individual European countries on the basis of reported carcinogenic effects in animal studies. In USA, a ban of specific colorants in cosmetic preparations is seldom used, while US FDA requires that the label should contain information on possible carcinogenic effects. Another difference between the use of hair colorants in USA and EU is that it is more common in USA that hairdressers mix colorants themselves. Under such conditions, the control of the dyes used and their purity is limited.

Risk assessment
At present, the SCCP is not in the position to finally assess the risk of reactions products of oxidative hair dyes due to the incomplete dossier. General conclusions regarding exposure to hair dye reaction products will be drawn after the complete results of the additional ongoing/planned *in vitro* dermal absorption studies have been submitted and evaluated. Based on robust data on exposure a final decision can be made if this exposure to reaction products is considered relevant from a toxicological point of view. If a relevant exposure to reaction products from hair dyeing cannot be excluded further testing on genotoxicity will be required to exclude the genotoxicity/mutagenicity potential of the reaction products of oxidative hair dye.

4. CONCLUSION

Qualitative and/or quantitative analyses of reaction products formed by various combinations of seven precursors and ten couplers, all in all 27 combinations, have been performed under conditions simulating hair dyeing. The reaction products of oxidative coupling were in agreement with the theoretical predictions based on the reaction kinetics. Substances used represented the highest tonnage precursors and couplers with a variety of substituents. The content of reaction products in the oxidative hair dyes under conditions simulating hair dyeing, was found to be 0.02-0.65%. However, the concentration of some active ingredients used for the formation of reaction products was not a worst case scenario according to maximum allowed concentrations of these ingredients. Studies on less than half of the studied combinations of precursors and couplers were submitted for evaluation, while for the remaining combinations only the results were reported.

Physico-chemical properties of 9 reaction products were described, and *in vitro* dermal absorption studies for these were performed at 1% in hair dye formulations in the presence

or absence of peroxide developer. *In vitro* dermal absorption of four of these reaction products were also studied at lower concentrations. It is reported that similar studies are either underway or are planned on 13 additional representative reaction products of oxidative hair dyes.

Reaction products of chloro- and nitro- substituted precursors and couplers of oxidative hair dyes have not been studied so far; and they should also be investigated in a similar way as other reaction products of precursors and couplers of oxidative hair dyes.

The epidemiological assessment has been concentrated on bladder cancer and haematological malignancies since no evidence was found linking personal use of hair dyes to a cancer risk at other sites.

* Although the published data are conflicting, especially when all types of hair dyes is considered, it is concluded that some studies indicate excess risks for chronic lymphoid leukaemia/small lymphocytic lymphoma and acute leukaemia for users of hair dyes.
* It is concluded that there is an indication of excess risk of bladder cancer for women in USA using permanent hair dyes frequently and for long time.
* The risk of developing a malignant disease in relation to personal use of hair dye is primarily related to users starting to dye their hair before 1980 although an increase in risk in women starting use after 1980 cannot be excluded.

The proposal to use the threshold of toxicological concern (TTC) approach or the margin of exposure (MoE) concept for the risk assessment of oxidative hair dyes can not be considered at this stage since the knowledge of exposure is a prerequisite. In addition, it has to be demonstrated that the available toxicity database of chemical compounds contains compounds similar to reaction products of oxidative hair dyes with regard to structural elements and complexity. Such a database should be established first. Furthermore, a detailed discussion of the structural elements of the hair dye reaction products with regard to structural alerts of genotoxicity and systemic toxicity is needed before the application of the TTC approach could be envisaged.

At present, the SCCP is not in the position to finally assess the risk of reactions products of oxidative hair dyes due to the incomplete dossier. General conclusions regarding exposure to hair dye reaction products will be drawn after submission and evaluation of the complete results of the additional ongoing/planned *in vitro* dermal absorption studies. Based on robust data on exposure a final decision can be made if the exposure to these substances is considered relevant from a toxicological point of view. If a relevant exposure to reaction products from hair dyeing cannot be excluded, further testing on genotoxicity will be required to exclude a genotoxicity/mutagenicity potential of the reaction products of oxidative hair dye.

5. MINORITY OPINION

Not applicable

6. REFERENCES

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