



EUROPEAN COMMISSION  
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment  
C7 - Risk assessment

## SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

### SCCP

**Skin penetration of oxidative hair dyes formed by the coupling of precursors and couplers under simulated conditions of hair dyeing**

**Update of the Annex to the**

**Opinion on Exposure to reactants and reaction products of oxidative hair dye formulations  
(doc. n° SCCP/0941/05)**

Adopted by the SCCP  
during the 8<sup>th</sup> plenary of 20 June 2006

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## 1. BACKGROUND

Commission services together with Member States agreed in April 2003 on a detailed programme of an overall strategy for the evaluation of hair dyes within the framework of the Cosmetics Directive 76/768/EEC. The strategy was published as *Information note on the use of ingredients in permanent and non-permanent hair dye formulations (dye precursors and direct dyes)* on:

<http://pharmacos.eudra.org/F3/cosmetic/doc/HairDyeStrategyInternet.pdf>.

This strategy has been decided following two opinions of the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP). In its opinion of June 2001 on "The Use of Permanent Hair Dyes and Bladder Cancer Risk" SCCNFP/484/01, the SCCNFP concluded that *the potential risk for the development of bladder cancer in past users of permanent hair dyes is of concern*.

The SCCNFP recommended that *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 and 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 its opinion of December 2002 on "Assessment Strategies for Hair Dyes" SCCNFP/0553/02, 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.

Against this background, Commission services together with Member States agreed on a regulatory strategy to all hair dyes in use. 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 have to be submitted by industry at the latest December 2007 for the evaluation in Scientific Committee on Consumer Products (SCCP). On 23 April 2004, SCCNFP forwarded its opinion on "Ring Study on Reaction Products from Typical Combinations of Hair Colouring Ingredients". It stated *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 is validated. However, both qualitative and quantitative information on reactants and reaction products of various hair dye formulations, under use conditions, are necessary for safety evaluation of these products*.

Meanwhile, COLIPA (European Cosmetics Toiletry and Perfumery Association) has submitted a "Technical Report Addressing Concerns regarding Exposure to Reaction Products During their Hair Dyeing Process" (January 2005) aiming at providing additional information for combinations of ingredients in hair dyes.

## 2. TERMS OF REFERENCE

1. Does the Scientific Committee on Consumer Products (SCCP) share the view presented in the “Technical Report” that oxidative hair dye reaction products pose no or negligible risk to human health?
2. If not, the SCCP is invited to identify any additional information necessary to evaluate the overall risk to reaction products of hair dyes.

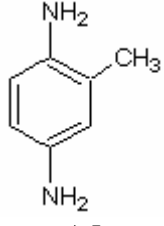
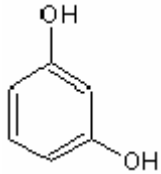
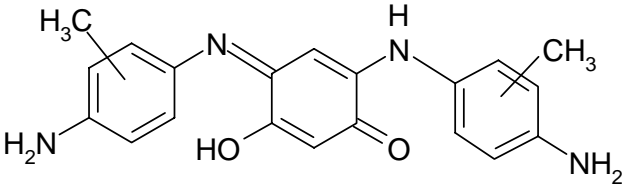
## 3. OPINION

### Introduction

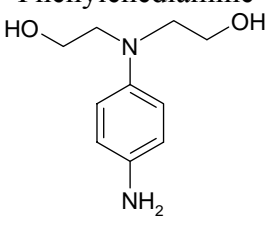
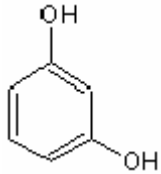
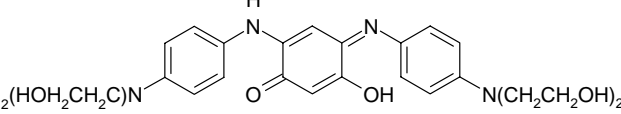
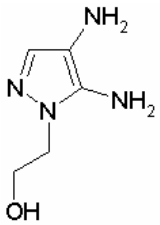
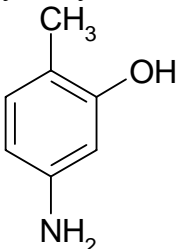
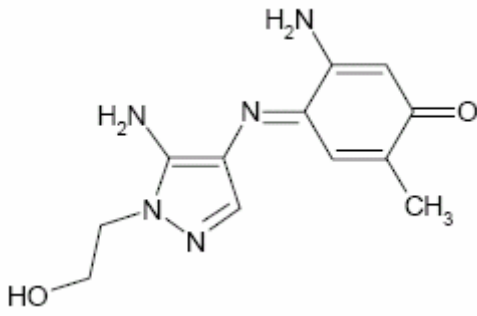
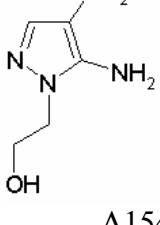
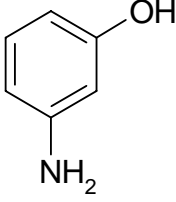
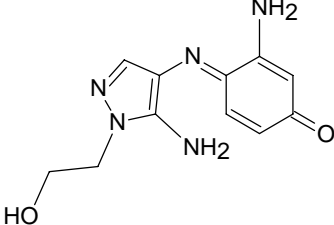
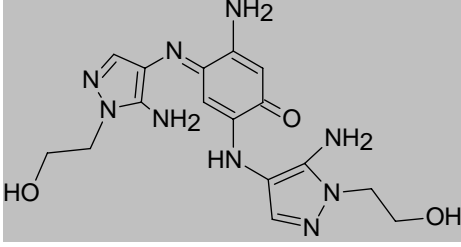
For the exposure assessments of precursor(s) and coupler(s) of oxidative hair dyes, as well as of the hair dyes formed, SCCNFP/SCCP has evaluated earlier submitted COLIPA reports on reactants and reaction products of oxidative hair dyes with respect to 1) approval of the methodology for the determination of unreacted precursors and couplers under the simulated conditions of hair dyeing (SCCNFP/0808/04), and 2) identification and determination of the oxidative hair dyes formed employing 11 precursor-coupler combinations in the presence of hydrogen peroxide (SCCP/0941/05). Furthermore, *in vitro* percutaneous absorption studies of oxidative hair dyes formed by the 4 precursor-coupler combinations were evaluated for exposure assessment (SCCP/0941/05).

In the second report on skin penetration of reaction products, physicochemical properties and *in vitro* percutaneous absorption studies of additional five oxidative hair dyes, derived from five different combinations of precursors (n = 4) and couplers (n = 3) have been submitted (Table 1). In the following, this submission has been evaluated with regard to exposure assessment of oxidative hair dyes including those reported earlier (“Technical Report Addressing Concerns regarding Exposure to Reaction Products During their Hair Dyeing Process” (January 2005))

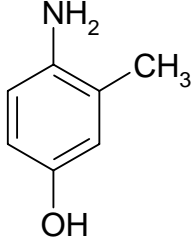
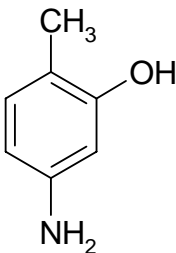
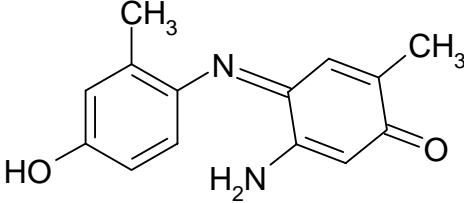
Table 1: Oxidative hair dyes (reaction products) tested for dermal absorption

Precursor	Coupler	Reaction Product (Hair dye)
Toluene-2,5-diamine  A5	Resorcinol  A11	 2-((4-amino-3-methylphenyl)amino)-4-((4-amino-3-methylphenyl)imino)-5-hydroxy-2,5-cyclohexadien-1-one <b>TrimerA5-A11-A5 (four isomers)</b>

Addendum to the  
opinion on exposure to reactants and reaction products of oxidative hair dye formulations

Precursor	Coupler	Reaction Product (Hair dye)
<p>N,N-bis(2-hydroxyethyl)-p-Phenylenediamine</p>  <p>A50</p>	<p>Resorcinol</p>  <p>A11</p>	 <p>2-[[4-bis-(2-hydroxyethyl)amino]-phenyl]amino]-4-[[4-bis-(2-hydroxyethyl)amino]-phenyl]imino]-5-hydroxy-2,5-cyclohexadien-1-one</p> <p><b>Trimer A50-A11-A50</b></p>
<p>1-Hydroxyethyl-4,5-diaminopyrazole</p>  <p>A154</p>	<p>4-Amino-2-hydroxytoluene</p>  <p>A27</p>	 <p>5-Amino-4-[5-amino-1-(2-hydroxyethyl)-1H-pyrazol-4-yl]imino]-2-methyl-2,5-cyclohexadien-1-one</p> <p><b>Dimer A154-A27</b></p>
<p>1-Hydroxyethyl-4,5-diaminopyrazole</p>  <p>A154</p>	<p><i>m</i>-Aminophenol</p>  <p>A15</p>	 <p>3-Amino-4-((5-amino-1-(2-hydroxyethyl)-1H-pyrazol-4-yl)imino)-2,5-cyclohexadien-1-one</p> <p><b>Dimer A154-A15</b></p>  <p>(4Z)-5-amino-2-{{5-amino-1-(2-hydroxyethyl)-1H-pyrazol-4-yl]amino}-4-{{5-amino-1-(2-hydroxyethyl)-1H-pyrazol-4-yl]imino}-2,5-cyclohexadien-1-one</p> <p><b>Trimer A154-A15-A154</b></p>

Addendum to the  
opinion on exposure to reactants and reaction products of oxidative hair dye formulations

Precursor	Coupler	Reaction Product (Hair dye)
4-amino- <i>m</i> -cresol  A74	4-Amino-2-hydroxytoluene  A27	 <b>Dimer A74-A27</b> 5-Amino-4-[(4-hydroxy-2-methylphenyl)imino]-2-methyl-2,5-cyclohexadien-1-one

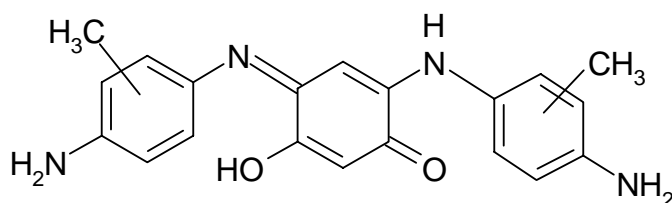
**Shaded area:** Physicochemical properties as well as dermal absorption of the trimer A154-A15-A154 are not submitted, and therefore, this trimer will not be discussed in this document.

## Physicochemical characterisation

**Hair dye:** Trimer A5-A11-A5

**Chemical name:** 2-[(4-amino-3-methylphenyl)amino]-4-[(4-amino-3-methylphenyl)imino]-5-hydroxy-2,5-cyclohexadien-1-one

**Chemical Structure:**



**CAS:** Not registered

**EINECS:** Not registered

**Batch:** SOB01135/3A

**Empirical formula:** C<sub>20</sub>H<sub>20</sub>N<sub>4</sub>O<sub>2</sub>

**Molecular weight:** 348.41

## Chemical characterisation and physico-chemical properties

Identification by NMR, IR, MS, and elemental analysis

UV/Vis spectrum:  $\lambda_{\max}$  at 208 nm, 244 nm, 324 nm, 454 nm and 608 nm in ethanol

Purity: the substance is a mixture of 4 isomers (3 major isomers) with purity > 96%, determined by HPLC

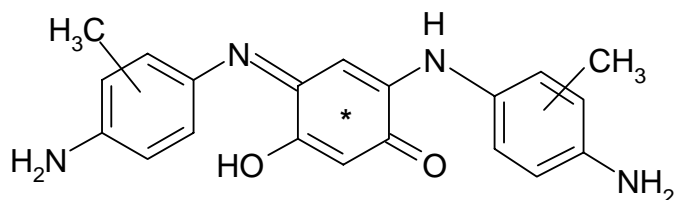
NMR content 81%. The content (area %) of the three isomers determined by HPLC-UV/Vis and HPLC-MS is described in the table below.

Addendum to the  
opinion on exposure to reactants and reaction products of oxidative hair dye formulations

Isomer	HPLC retention time, min	HPLC area % at		
		250 nm	600 nm	MS
1	4.2-4.38	14.4	9.7	24.8
2	4.7-4.93	55.7	55.0	43.5
3	5.4-5.66	29.95	35.35	27.4

Appearance: Black powder  
 Melting point: 240-243 °C  
 Solubility: 0.08% (w/w) in water  
 >10% (w/w) in DMSO

**Identification and properties of <sup>14</sup>C-labelled test substance A5-A11-A5 used for *in vitro* percutaneous absorption**

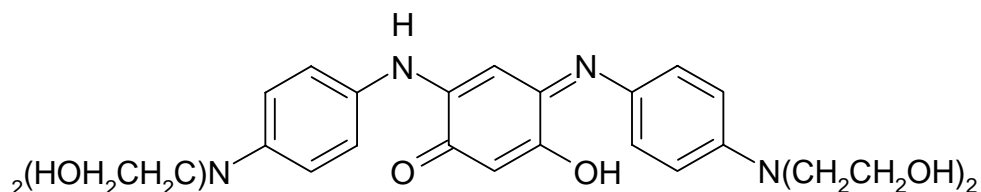


Lot No.: 445-175-056  
 Radiochemical purity: 97.1% by HPLC, based on combined trimer

**Hair dye:** Trimer A50-A11-A50

**Chemical name:** 2-[[4-[bis(2-hydroxyethyl)amino]-phenyl]amino]-4-[[4-[bis(2-hydroxyethyl)amino]-phenyl]imino]- 5-hydroxy-2,5-cyclohexadien-1-one

**Chemical Structure:**



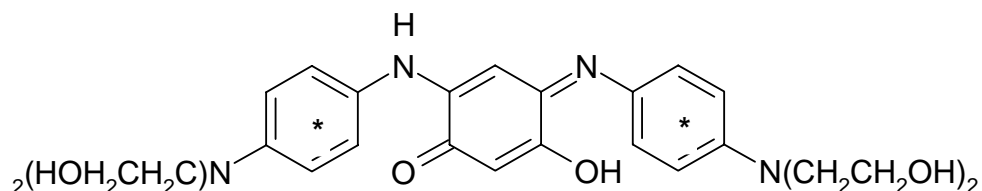
**CAS:** Not registered  
**EINECS:** Not registered  
**Batch:** PG62-105-1  
**Empirical formula:** C<sub>26</sub>H<sub>33</sub>N<sub>5</sub>O<sub>5</sub>  
**Molecular weight:** 495.6

### Chemical characterisation and physico-chemical properties

Identification by NMR, IR, MS, and elemental analysis

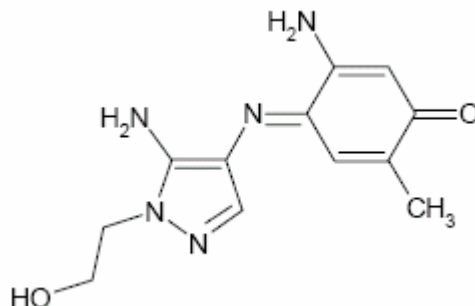
UV/Vis spectrum:  $\lambda_{\max}$  521 nm  
Purity: 97.1% (HPLC)  
Appearance: Black powder  
Melting point: 209-212°C  
Weight loss: 1.07% at 110°C, for 2 h  
Solubility: < 0.5% (w/w) in water  
0.5% (w/w) in ethanol  
1.50% (w/w) in DMSO

### Identification and properties of <sup>14</sup>C-labelled test substance A50-A11-A50 used for *in vitro* percutaneous absorption



Lot No.: 156-060-056  
Radiochemical purity: 99.5% by HPLC



**Hair dye:** Dimer A154-A27**Chemical name:** 5-amino-4-[5-amino-1-(2-hydroxyethyl)-1H-pyrazol-4-yl]imino]-2-methyl-2,5-cyclohexadien-1-one**Chemical Structure:****CAS:** Not registered**EINECS:** Not registered**Batch:** AMA0112/4A**Empirical formula:** C<sub>12</sub>H<sub>15</sub>N<sub>5</sub>O<sub>2</sub>**Molecular weight:** 261.29**Chemical characterisation and physico-chemical properties**

Identification by NMR, MS, and elemental analysis

UV/Vis spectrum:  $\lambda_{\max}$  at 208 nm, 330 nm and 476 nm in ethanol

Content: 91.5% (w/w) by NMR

Water content: Ca. 7% (w/w)

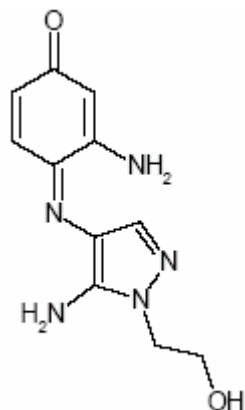
Methanol content: Ca. 1.1% (w/w)

Appearance: Red powder

Melting point: 234-237°C

Solubility: 0.2% (w/w) in water, pH 2.8

&gt;15% (w/w) in DMSO

**Hair dye:** Dimer A154-A15**Chemical name:** 3-amino-4-[5-amino-1-(2-hydroxyethyl)-1H-pyrazol-4-yl]imino]-2-methyl-2,5-cyclohexadien-1-one**Chemical Structure:**

**CAS:** Not registered  
**EINECS:** Not registered  
**Batch:** AMA0113/11A  
**Empirical formula:** C<sub>11</sub>H<sub>13</sub>N<sub>5</sub>O<sub>2</sub>  
**Molecular weight:** 247.26

**Chemical characterisation and physico-chemical properties**

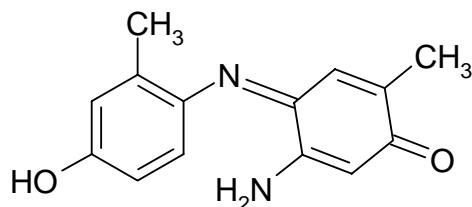
Identification by NMR, MS, and elemental analysis

UV/Vis spectrum:  $\lambda_{\max}$  at 208 nm, 336 nm and 486 nm in ethanol  
 Content: 90.0% (w/w) by NMR  
 HPLC purity: 99.4% (HPLC peak area at 488 nm)  
 Water content: Ca. 4.7% (w/w)  
 Methanol content: Ca. 2.4% (w/w)  
 Appearance: Black-brown powder  
 Melting point: 211.9-213.6 °C  
 Solubility: 0.06% (w/w) in water, pH 8.0  
 0.1% (w/w) in acetone  
 > 8% (w/w) in DMSO

**Hair dye:** Dimer A74-A27

**Chemical name:** 5-amino-4-[(4-hydroxy-2-methylphenyl)imino]-2-methyl-2,5-cyclohexadien-1-one

**Chemical Structure:**



**CAS:** Not registered

**EINECS:** Not registered

**Batch:** SO-1100.27

**Empirical formula:** C<sub>14</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>

**Molecular weight:** 242.28

### Chemical characterisation and physico-chemical properties

Identification by NMR, MS

Content 96.0% (w/w) by NMR

HPLC purity: 97.6% (HPLC peak area)

Appearance: Dark red powder

Melting point: 120-125°C

Solubility: 0.11% (w/w) in water, pH 8.0

Ca. 10% (w/w) in DMSO

## ***In vitro* percutaneous absorption**

### **A5-A11-A5 Trimer**

2-[(4-amino-3-methylphenyl)amino]-4-[(4-amino-3-methylphenyl)imino]-5-hydroxy-2,5-cyclohexadien-1-one

Guideline:	OECD 428
Test material:	Cream formulation (koleston base) containing ca. 2% <sup>14</sup> C-A5-A11-A5 trimer diluted 1:1 with a Welloxon peroxide developer or Welloxon placebo developer
Skin samples:	12 human dermatomed skin membranes, thickness ca. 400 nm
Receptor fluid:	Phosphate buffered saline (PBS) containing 4% polyoxyethylene 20 oleyl ether Solubility of A5-A11-A5 trimer in the receptor fluid: 0.0016% (w/w)
GLP:	in compliance

Cream formulations, diluted with either a peroxide developer or a placebo developer, were applied on human skin membranes (2.54 cm<sup>2</sup>) mounted in glass diffusion cells (static) at nominal rate of 20 mg/cm<sup>2</sup>. After a contact period of 30 min, the dose was washed from the surface of the skin. Samples of the receptor fluid were taken at recorded time intervals over a 48 h period, during which time the applications remained unoccluded. At the end of the experiment, the surface of the skin was washed and layers of *stratum corneum* removed by tape stripping. The content of A5-A11-A5 trimer in the receptor fluid, dermis/epidermis, tape strips and all washings was measured by scintillation counting. Skin integrity was checked by measurement of the electrical resistance across the skin membrane

### Results

Results from one of the experiments in the presence of peroxide developer were excluded because the skin was damaged.

Trimer A5-A11-A5 penetration in the skin (receptor fluid + dermis/epidermis) in the presence of peroxide developer:

0.035 - 0.136 µg/cm<sup>2</sup> (0.0767 ± 0.027 µg/cm<sup>2</sup>)

Total recovery: 91.7- 102.0 %

Trimer A5-A11-A5 penetration in the skin (receptor fluid + dermis/epidermis) in the presence of placebo developer:

0.023 - 0.221 µg/cm<sup>2</sup> (0.092 ± 0.054 µg/cm<sup>2</sup>)

Total recovery: 94.5 - 110.0%

### Comments

The number of skin donors, identification of skin donors (age/sex) and part(s) of the body from which skin were taken is not reported. The compositions of Koleston base, Welloxon peroxide developer and placebo developer are not reported. Method performance could not be assessed as the data on positive controls is not reported.

**A50-A11-A50 Trimer**

2-[[4-[bis(2-hydroxyethyl)amino]-phenyl]amino]-4-[[4-[bis(2-hydroxyethyl)amino]-phenyl]imino]-5-hydroxy-2,5-cyclohexadien-1-one

Guideline: OECD 428  
 Test material: Cream formulation (Koleston base) containing ca. 2% <sup>14</sup>C-A50-A11-A50 trimer diluted 1:1 with a Welloxon peroxide developer  
 Skin samples: 12 human dermatomed skin membranes, thickness ca. 400 nm  
 Receptor fluid: Phosphate buffered saline (PBS) containing 4% polyoxyethylene 20 oleyl ether  
 Solubility of A50-A11-A50 trimer in the receptor fluid: 0.0016% (w/w)  
 GLP: in compliance

Cream formulation diluted with a peroxide developer was applied on human skin membranes (2.54 cm<sup>2</sup>) mounted in glass diffusion cells (static) at nominal rate of 20 mg/cm<sup>2</sup>. After a contact period of 30 min, the dose was washed from the surface of the skin. Samples of the receptor fluid were taken at recorded time intervals over a 48 h period, during which time the applications remained unoccluded. At the end of the experiment, the surface of the skin was washed and layers of *stratum corneum* removed by tape stripping. The content of A50-A11-A50 trimer in the receptor fluid, dermis/epidermis, tape strips and all washings was measured by scintillation counting. Skin integrity was checked by measurement of the electrical resistance across the skin membrane

**Results**

Trimer A50-A11-A50 penetration in the skin (receptor fluid + dermis/epidermis) in the presence of peroxide developer:

0.012 - 0.073 µg/cm<sup>2</sup> (0.032 ± 0.023 µg/cm<sup>2</sup>)

Total recovery: 94.4 - 105.0%

**Comments**

The number of skin donors, identification of skin donors (age/sex) and part(s) of the body from which skin were taken is not reported. The compositions of Koleston base and Welloxon peroxide developer are not reported. Method performance could not be assessed as the data on positive controls is not reported.

**Dimer A154-A27**

5-amino-4-[5-amino-1-(2-hydroxyethyl)-1H-pyrazol-4-yl]imino]-2-methyl-2,5-cyclohexadien-1-one

Guideline: OECD 428  
 Test material: Cream formulation (SOB0452/2A) containing ca. 1% A154-A27 dimer  
 Skin samples: 12 porcine ear dermatomed skin; thickness ca. 300 nm; 12 donors  
 Receptor fluid: Saline (0.9% NaCl), stability of A154-A27 dimer in the saline: 6 days  
 GLP: in compliance

Two independent experiments were performed on fresh dermatomed pig skin samples mounted on diffusion cells between donor and receptor chambers. Sample application area was 1 cm<sup>2</sup>. The blank samples of receptor fluid were collected immediately after filling the receptor chamber. 20 µl of the cream formulation was applied to each skin sample for 30 min and then washed off

using shampoo and deionised water. The penetration was monitored for 24 h under non-occluded conditions. Then *stratum corneum* was separated from dermis/epidermis by tape stripping. Amounts of A154-A27 dimer in receptor fluid and in saline extracts of dermis/epidermis, *stratum corneum*, tape and washings were determined by HPLC, having 1 ng/ml detection limit for the A154-A27 dimer.

The integrity of the skin membranes was checked by measuring the conductivity across the membrane. Positive control data for benzoic acid, caffeine and testosterone are reported.

#### Results

Dimer A154-A27 penetration in the skin (receptor fluid + dermis/epidermis): 0.006 - 0.035  $\mu\text{g}/\text{cm}^2$  ( $0.009 \pm 0.008 \mu\text{g}/\text{cm}^2$ ). Total recovery:  $92.24 \pm 1.58 \%$

#### Dimer A154-A15

3-amino-4-[5-amino-1-(2-hydroxyethyl)-1H-pyrazol-4-yl]imino]-2-methyl-2,5-cyclohexadien-1-one

Guideline: OECD 428

Test material: Cream formulation (SOB0452/2A) containing ca. 1% A154-A15 dimer

Skin samples: 12 porcine ear dermatomed skin, thickness ca. 300 nm; 12 donors

Receptor fluid: Saline (0.9% NaCl), stability of A154-A15 dimer in the saline: 6 days

GLP: in compliance

Two independent experiments were performed on fresh dermatomed pig skin samples mounted on diffusion cells between donor and receptor chambers. Sample application area was 1  $\text{cm}^2$ . The blank samples of receptor fluid were collected immediately after filling the receptor chamber. 20  $\mu\text{l}$  of the cream formulation was applied to each skin sample for 30 min and then washed off using shampoo and deionised water. The penetration was monitored for 24 h under non-occluded conditions. Then *stratum corneum* was separated from dermis/epidermis by tape stripping. Amounts of A154-A15 dimer in receptor fluid and in saline extracts of dermis/epidermis, *stratum corneum*, tape and washings were determined by HPLC, having 1 ng/ml detection limit for the A154-A15 dimer.

The integrity of the skin membranes was checked by measuring the conductivity across the membrane. Positive control data for benzoic acid, caffeine and testosterone are reported.

#### Results

Dimer A154-A15 penetration in the skin (receptor fluid + dermis/epidermis): 0.020 - 0.012  $\mu\text{g}/\text{cm}^2$  ( $0.006 \pm 0.044 \mu\text{g}/\text{cm}^2$ ). Total recovery:  $108.66 \pm 5.89 \%$

#### Dimer A74-A27

5-amino-4-[(4-hydroxy-2-methylphenyl)imino]-2-methyl-2,5-cyclohexadien-1-one

*Report only on analytical method was available. Report on percutaneous absorption study is not available. Following is copied from the COLIPA report of October 2005: "Technical report addressing concerns regarding exposure to reaction products during the hair dyeing process"*

*Experiments were performed in duplicate (2 x 6 cells). 20  $\mu\text{l}$  (approximately 20 mg) of a formulation containing 1% of the reaction product (Dimer A74-A27) were applied without*

hydrogen peroxide to approximately 1 cm<sup>2</sup> of excised and dermatomed abdominal pig skin (thickness 400-500 µm), resulting in an administration of 140 µg reaction product/ cm<sup>2</sup>. Due to the small scale of the experiment and the fact that the formulation was aerated, the applied amount of product was slightly lower than the targeted 200 µg/ cm<sup>2</sup>. The receptor fluid was physiological sodium chloride solution (0.9% NaCl). The presence of absorbed reaction product was determined by HPLC. The limit of quantification of the HPLC based analytical method was 7.54 ng/ml.

### Results

Dermal absorption (receptor fluid+ dermis/epidermis): 0.02 ± 0.001 µg/ cm<sup>2</sup>.

Recovery: 95.00 ± 5.63%

### Comments

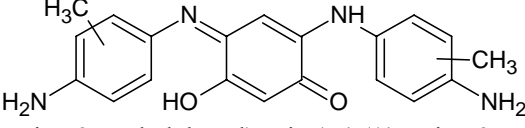
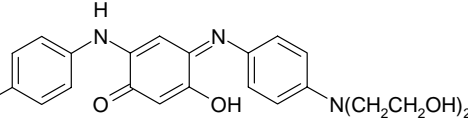
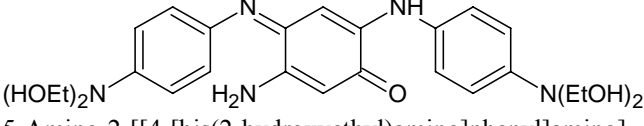
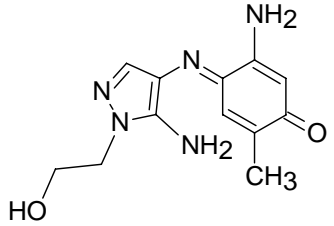
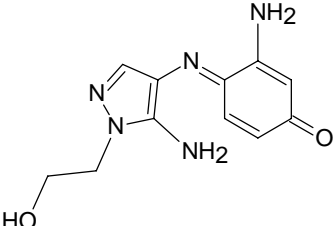
Results of this study can not be accepted without checking the quality of the study.

## Discussion

Studies of five oxidative hair dyes (reaction products of precursors and couplers in the presence of hydrogen peroxide) formed under simulated conditions of hair dyeing are reported in the present submission. The characterisation of the hair dyes is adequate and the physico-chemical properties of the hair dyes are acceptable for the purpose of the evaluation of *in vitro* percutaneous absorption studies. The log P<sub>ow</sub> of the hair dyes would have been helpful for the evaluation. The analytical methods used are adequate. The percutaneous absorption study of one of the hair dyes, A74-A27 dimer, was not submitted. Hence the reported results of this study will not be considered in the following discussion.

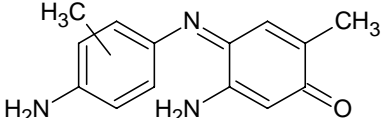
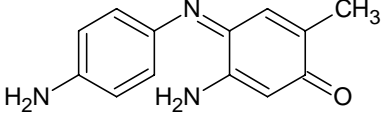
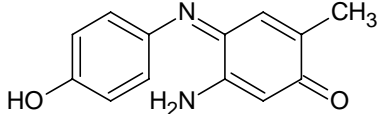
The *in vitro* percutaneous absorption of the hair dyes (reaction products) is studied using either dermatomed human or pig skin, where <sup>14</sup>C-labelled reaction products or unlabelled reaction products have been used as test substances. The hair dye (cream) formulations containing 1% reaction products (final concentration), with or without hydrogen peroxide, were used as test materials. 1% concentration of reaction products in the test materials is more than that would be expected from the kinetics of oxidative hair dye formation under simulated hair dyeing conditions. Thus, the concentrations of hair dyes used in the formulations may be considered as worst case. The maximum observed dermal absorption rates of the oxidative hair dyes (both investigated in the present investigation as well as in the previous submission), under the respective experimental conditions, are described in Table 2.

Table 2: Maximum observed dermal absorption of oxidative hair dyes *in vitro*

Oxidative hair dye (reaction product of precursor and coupler in the presence of hydrogen peroxide)	Maximum observed dermal absorption <i>in vitro</i> $\mu\text{g}/\text{cm}^2$
 <p>2-((4-amino-3-methylphenyl)amino)-4-((4-amino-3-methylphenyl)imino)-5-hydroxy-2,5-cyclohexadien-1-one</p> <p><b>Trimer A5-A11-A5</b></p>	<p>0.221</p> <p>Test performed using human skin and in the presence of hydrogen peroxide</p>
 <p>5-Hydroxy-2-[[4-bis-(2-hydroxyethyl)amino]-phenyl]amino]-4-[[4-bis-(2-hydroxyethyl)amino]-phenyl]imino]-2,5-cyclohexadien-1-one</p> <p><b>Trimer A50-A11-A50</b></p>	<p>0.073</p> <p>Test performed using human skin and in the presence of hydrogen peroxide</p>
 <p>5-Amino-2-[[4-[bis(2-hydroxyethyl)amino]phenyl]amino]-4-[[4-[bis(2-hydroxyethyl)amino]phenyl]imino]-2,5-cyclohexadien-1-one</p> <p><b>Trimer A50-A15-A50</b></p>	<p>0.182</p> <p>Test performed using human skin and in the presence of hydrogen peroxide</p>
 <p>5-Amino-4-[5-amino-1-(2-hydroxyethyl)-1H-pyrazol-4-yl]imino]-2-methyl-2,5-cyclohexadien-1-one</p> <p><b>Dimer A154-A27</b></p>	<p>0.035</p> <p>Test performed using pig skin and in the absence of hydrogen peroxide</p>
 <p>3-Amino-4-((5-amino-1-(2-hydroxyethyl)-1H-pyrazol-4-yl)imino)-2,5-cyclohexadien-1-one</p> <p><b>Dimer A154-A15</b></p>	<p>0.012</p> <p>Test performed using pig skin and in the absence of hydrogen peroxide</p>



Addendum to the  
opinion on exposure to reactants and reaction products of oxidative hair dye formulations

 <p>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</p> <p style="text-align: center;"><b>Dimer A5-A27</b></p>	<p>0.157</p> <p>Test performed using pig skin and in the absence of hydrogen peroxide</p>
 <p>5-Amino-4-((4-aminophenyl)imino)-2-methyl-2,5-cyclohexadien-1-one</p> <p style="text-align: center;"><b>Dimer A7-A27</b></p>	<p>0.012</p> <p>Test performed using human skin and in the presence of hydrogen peroxide</p>
 <p>5-Amino-4-((4-hydroxyphenyl)imino)-2-methyl-2,5-cyclohexadien-1-one</p> <p style="text-align: center;"><b>Dimer A16-A27</b></p>	<p>0.271</p> <p>Test performed using human skin and in the absence of hydrogen peroxide</p>

The dermal absorption of the eight investigated hair dyes varied from 0.012 -0.271  $\mu\text{g}/\text{cm}^2$ . In the worst case scenario of 1% hair dye exposure to 700  $\text{cm}^2$  (scalp), 0.14 – 3.16  $\mu\text{g}/\text{kg}$  bw of the hair dye will be absorbed. Depending upon the toxic profile of the hair dyes, some of these may be of concern.

The SCCP understands that about a hundred different precursors and couplers are used in the oxidative hair dye formulations in EU. Studies, similar to those presented here, should be extended to include more indicative combinations of precursors and couplers. According to the updated strategy of hair dyes (genotoxicity, doc. n° SCCP/0971/06) further testing may be required.

#### 4. CONCLUSION

The SCCP understands that about a hundred different precursors and couplers are used in the oxidative hair dye formulations in EU. The data presented indicates that in some cases significant amounts of oxidative hair dye reaction products become systemically available to the consumer.

Studies, similar to those presented here, 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) further testing may be required.

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

## 5. MINORITY OPINION

Not applicable

## 6. REFERENCES

1. SCCNFP/0808/04: Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers concerning a ring study on reaction products from typical combinations of hair colouring ingredients, adopted by the SCCNFP on 23 April 2004.
2. SCCP/0941/05: Opinion on Exposure to reactants and reaction products of oxidative hair dye formulations, adopted by the SCCP on 13 December 2005.
3. SCCP/0971/06: Updated recommended strategy for testing oxidative hair dye substances for their potential mutagenicity/genotoxicity (SCCP'S Notes of Guidance), adopted by the SCCP on 28 March 2006.

## 7. ACKNOWLEDGEMENTS

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