SAFETY OF HAIR DYES USE. TOXICOLOGICAL EXPOSURE

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Summary

In recent years hair colouring products popularity has been confronted with the safety of its use. Proposed in 2001 European Union strategy aimed to distinguish the safety dyes list has brought first results. 22 dyes have been banned to use in hair colouring products because of their non-complete toxicological dossiers. Currently, 117 dyes and their precursors are thoroughly assessed including their toxic properties and bioavailability in simulated use conditions.

With a wide-spread research, in literature more and more information on skin penetration ability of dyeing compounds appear, but these data concern particular dyes permeability without any attempts to determine the dependence between penetration ability and dye structure. That kind of relationships are of a great importance from practical site – the isolation of the compounds, which bring desired colour effects, with high affinity to hair and minimal to the skin, are crucial in the viewpoint of effective colouring at maximal safety of application.

The investigation of penetration ability of a series of semi-permanent dyes from the nitrophenylene-diamines and nitroaminophenols groups was carried out. The research indicates different penetration ability depending on the molecular structure of investigated dyes. Diffusion inside the stratum corneum-like lipid liquid-crystalline structures is a limiting factor for permeation.

Riassunto

In questi ultimi anni la grande popularità dei coloranti per capelli ed il loro frequente uso è stato valutato per determinarne la sicurezza.

La proposta di legge della Comunità Europea intesa ad evidenziare la sicurezza d’uso dei coloranti per capelli ha dato i primi risultati ponendone al bando 22 i cui dati tossicologici non sono stati ritenuti sufficienti.
Ai giorni nostri 117 coloranti con i relativi precursori sono stati classificati e controllati sotto l’aspetto tossicologico nelle normali condizioni d’uso. Molti sono i dati presenti nella letteratura anche riguardanti la penetrabilità di questi composti in rapporto alla loro struttura chimica. Questo tipo di relazione risulterebbe molto importante soprattutto per determinarne la massima affinità nei confronti della struttura dei capelli con la minima affinità nei confronti della cute. La conoscenza più approfondita del loro comportamento darebbe la possibilità di determinare l’affinità che il colorante possiede nei confronti della cheratina dei capelli contribuendo anche alla loro massima sicurezza d’uso durante l’applicazione. Con questo studio si è voluto determinare il grado di penetrabilità di una serie di coloranti semi permanenti appartenenti al gruppo delle nitrofenilenediamine e dei nitroaminofenoli. E’ stato così dimostrato come la penetrazione attraverso la barriera cutanea dipenda in modo significativo dalla struttura chimica dei coloranti.
INTRODUCTION

In recent years the cosmetics consumers’ health safety assurance is one of the most often considered problem in circles of consumers’ organisations, science centres and also cosmetic manufacturers’.

In agreement with European Union legislative bodies cosmetic cannot be harmful for consumers. This attitude had begun thoroughly research in safety application of preservatives, UV filters, fragrances and others, for which literature reports shows even minimal usage adverse effects probability. In this field, a special place is taken by hair dyes used in hair colouring products.

Hair dyes and its precursors are a group of cosmetics ingredients with a various chemical structures. The presence of chromophore groups in a dye structure (or in case of oxidative dyeing - in structure of pigment synthesized during colouring process) is a basic parameter, which determines its hair colouring products usage. In case of the specific structure, a part of hair dyes could possess toxic properties.

HAIR DYES SAFETY APPLICATION ASSESSMENT

In relation to a large hair dyeing popularity confronted with potentially toxic properties of colouring compounds Scientific Committee on Consumer Products – SCCP begun an insightful analysis of particular hair dyes and it precursors properties (SCCNFP/0553/01). The scheme of proposed strategy is presented in Figure I.

According to incorporated hair dyes safety assessment strategy the fully responsiveness for products safety is transferred to dyes and cosmetics manufacturers. After consideration of the dyeing mixture application specificity, the results insufficiency from the toxicity research for particular substances has been stated (especially results carried out with in vitro methods, or these with the extrapolation from oral tests into skin application). With the SCCP opinion the toxicological analysis carried out with simulated use conditions is necessary; the time of application, product pH, used vehiculum (applied dyeing compounds and its precursors mixture) should be taken into account (SCCP/0959/05).

The necessity to consider the substance bioavailability and also possible metabolic transformations after skin application (dependently on the road of application metabolism could be different) had been recommended in the assessment of Margin of Safety (MOS).

In agreement with the schedule of hair dyes safety assessment strategy at July 2006 SCCP notify the list of 22 substances, which use in hair colouring products is banned since December 1st 2006. The list contains compounds for which any toxicological data had not been submitted (IP/06/1047). The ban for substances with indeterminate toxicological profiles is significant: the ban for something which is not known (not that which is harmful) is the beginning of the brand new approach for the problem of consumers’ health safety.

The dossier for 117 substances had been submitted to SCCP by the cosmetic industry. After thoroughly analysis its will be used to the positive list of safety hair dyes generation. Up to now SCCP publicize opinion on 47 (from the group of 117) substances, further will be successively determined (4).

Additionally SCCP notify the opinion on hair dyes possible mutagenesis/carcinogenesis (SCCP/0930/05, SCCNFP/0797/04), recommends the standard protocols to assess above mentioned hair dyes toxicity (SCCNFP/0720/03, final), and also publicize a memorandum about its sensitising potential (8).
1ST STEP: SUBMISSION OF SAFETY DATA

There is a safety file submitted or the substance is already regulated

No file ever submitted → BAN

Deadline: 30 Sept 2003

2ND STEP: SUBMISSION OF UPDATED/ADDITIONAL DATA

Individual safety files submitted - up to new SCCP requirements

No updated Submission → BAN

Evaluation by SCCP

Deadline: July 2005

PASSED → BAN

3RD STEP: COMBINATIONS OF OXIDATIVE HAIR DYES

Combination of ingredients files submitted

Deadline: December 2007

Evaluation by SCCP

PASSED

FAILED → BAN

Annex III, 1

POSITIVE LIST

Fig. 1 Hair dyes safety assessment strategy (SCCNFP/0553/01).
Such a multidirectional and a wide range research have not been conducted, yet. Currently the hair dyes and its precursors are the most completely analysed group of cosmetic ingredients.

**HAIR DYSES AND ITS PRECURSORS BIOAVAILABILITY**

The fundamental for hair dyes safety assessment is its bioavailability in the simulated use conditions. If substance, after skin application, does not have transdermal penetration ability, is in condition of typical hair colouring product application biologically unavailable. In such conditions, the potential toxic effects of its use cannot be observed.

The hair dyeing conditions are unique, the description of exposure during colouring process is presented below:

- Relatively short term of application (typically hair dyeing mixture is applied for 30-40 minutes)
- Hair provides a large surface area and competes with scalp for dyestuff absorption
- The cosmetic components could influence on dyes and its precursors penetration ability
- Substantial shade-related variations in applied dose
- Colouring products are mixtures of dyeing substances with different skin penetration ability, applied simultaneously

Additionally exposure specificity depends on the dyeing method, in case of oxidative products:

- Alkaline pH = 9-10 swollen the hair (allows for colour precursors penetration into the hair)
- Cosmetic ingredients (besides colouring substances) could be responsible for dyeing mixture adverse effects (especially in relation to sensitising potential; for example: alkalines, hydrogen peroxide)
- The reactions running in dyeing mixture, during the dyeing process, cause the decrease in the amount of colour precursors – the substrates reacts each other, and the coloured pigments, responsible for colour effects after treatment, are synthesized – the amount of pigments and its chemical structures depends on the structures of used substrates and the reactions kinetics (SCCP/0941/05).

The compounds of oxidative hair colouring products are additionally analysed. Particular precursors and colour modifiers are tested alone, and also in mixtures typically used in market products. The oxidative reaction products are identified, its dermal penetration ability is estimated and its potentially toxic properties are also determined (SCCNFP/0808/04, SCCP/0941/05, SCCP/1004/06).

First data on hair dyes skin penetration comes from 70-ties last century. The urine discoloration where found for women who dyed hair. Since that moment hair dyes and its precursors transdermal penetration had been investigated in vivo, e.g. on human volunteers (where 14C radio labelled dyes and its metabolites where identified in urine and other systemic fluids); or with in vitro methods, with model membranes or human, mammalian skin application.

**CHEMICAL STRUCTURE AND THE PENETRATION ABILITY**

Higher understanding in the field of the skin penetration allows predicting substance penetration ability and the determination of physicochemical parameters, which decide about it.

From the viewpoint of penetration through lipid liquid-crystalline structures of *stratum corneum*, transport is a complex process with several steps involved. The physicochemical processes taking parts in penetration process are as follows: compound partition between donor vehicle and a membrane (*stratum corneum*), next - the diffu-
sion inside the membrane. The final step is another partition between lipid membrane and hydrophilic receptor system (living layers of the skin).

The lipophilicity of a substance is the most often parameter used to predict permeation ability of a compounds in biological systems. It is usually expressed as a partition coefficient in octanol-water system (logPo/w) and describes substance affinity to each phase.

Lipophilicity is a determinant of compound partition between donor vehicle and a membrane (stratum corneum), and also decides about partition to more hydrophilic receptor system (living layers of the skin).

The penetration from lipophilicity dependence is parabolic, although the exceptions form this principle are known. Mostly increase in compound organic phase affinity cause increase in penetration ability, but up to some limit level. When molecule becomes too hydrophobic, its penetration to water phase comes slower and slower, and as an effect: the transport through membrane (stratum corneum) into receptor system (living layers of the skin) is hindered (Zatz, 1991; Guy and Hadgraft, 1993; Roberts et al., 1995; Pliška et al., 1996; Mäkkiä et al., 2004).

The penetration rate is determinates by the of diffusion processes inside a membrane (stratum corneum). The diffusion of molecule is connected with its size, electrostatic properties and ability to form intermolecular bonds (mainly H-bonds) (Pugh et al., 1996; du Plessis et al., 2001; Plessis et al., 2002; Abraham et al., 2002; Geinoz et al., 2002; Mäkkiä et al., 2004).

Hair dyes and its precursors penetration ability.

The data submitted SCCP includes, for particular substances used in dyeing mixtures, an information on hair dyes skin penetration ability. The penetration studies results for 47 hair dyes, performed in typical conditions of application (in accordance with SCCP guidelines), shows that only a small percent of applied doses permeate the horny layer (the presented information from SCCP given opinions concerns only the dyes examined up to now since the strategy incorporation). Less than 5% of applied doses were bioavailable both in oxidative and in semi-permanent (without hydrogen peroxide) conditions for most of analysed compounds (more than 95%). It has to be stated that a part of given results have not been used for Margin of Safety (MOS) calculations. The further research is needed for a part of hair dyes (4).

The aim of the current hair dyes safety assessment strategy is to distinguish the list of safety dyes. The ban for usage of some compounds is possible, and as a result the limitation in range of colours offered to consumers could be observed.
<table>
<thead>
<tr>
<th>INCI name</th>
<th>SCCP opinion</th>
<th>SCCP document</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-nitro-o-phenylenediamine (3a)</td>
<td>The use as an oxidative hair dye substance at a maximum concentration (Cmax) of 0.5% in the finished cosmetic product (after mixing with hydrogen peroxide) does not pose a risk to the health of the consumer, apart from its sensitising potential. Studies on genotoxicity/mutagenicity in finished hair dye formulations should be undertaken*</td>
<td>(SCCP/0980/06)</td>
</tr>
<tr>
<td>HC Red 1 (2a)</td>
<td>The use in semi-permanent hair dye formulation at Cmax = 1.0% does not pose a risk to the health of the consumer, apart from its sensitising property.</td>
<td>(SCCP/0981/06)</td>
</tr>
<tr>
<td>N,N'-bis (2-hydroxyethyl)-2-nitro-p-phenylenediamine (1c)</td>
<td>The information submitted is inadequate to assess the safe use of the substance, the following information is required: - chemical characterization - percutaneous absorption in an oxidative environment. - data on the genotoxicity/mutagenicity*</td>
<td>(SCCNFP/0781/04)</td>
</tr>
<tr>
<td>HC Blue 2 (1d)</td>
<td>Itself in semi-permanent hair dye formulations at Cmax = 2.8% in the finished cosmetic product does not pose a risk to the health of the consumer, apart from its sensitising potential. It is comprised of both a secondary and a tertiary amino group, and thus is prone to nitrosation**.</td>
<td>(SCCP/1035/06)</td>
</tr>
<tr>
<td>4-amino-3-nitrophenol (2a)</td>
<td>Extreme sensitiser. Insufficient information to assess the safe use of the substance, the following information is required: - an in vitro percutaneous absorption study should be performed*, - an additional mutagenicity/genotoxicity test should be performed* in order to exclude a gene mutation potential.</td>
<td>(SCCP/1059/06)</td>
</tr>
<tr>
<td>3-nitro-p-hydroxyethylaminophenol (4b)</td>
<td>The use itself as an oxidative hair dye substance at Cmax = 3.0% in the finished cosmetic product (after mixing with hydrogen peroxide) or as an ingredient in semi-permanent hair colouring products at Cmax = 1.85% does not pose a risk to the health of the consumer, apart from its sensitising potential. It is a secondary amine, and thus is prone to nitrosation**.</td>
<td>(SCCP/1036/06)</td>
</tr>
</tbody>
</table>

*performed following the relevant SCCNFP/SCCP opinions and in accordance with its Notes of Guidance.

** It should not be used in combination with nitrosating substances. The nitrosamine content should be < 50ppb.
Nitrophenylenediamines and nitroaminophenols penetration ability.

The analysis of particular substances used in hair colouring properties is unquestionably proper; but the dyes penetration ability with its physicochemical properties correlation seems to have practical meaning.

The penetration ability of several direct dyes used in hair dyeing had been investigated, as a model nitrophenylenediamine and nitroaminophenol derivatives were chosen. This group of compounds has been used in hair colouring products for a long time, due to its wide range of colours, low price, high stability and good keratin affinity. Table I shows current SCCP opinions on a few of analysed dyes.

Investigated dyes structures are presented in Figure 2, the dyes names are given according to INCI nomenclature. (For a better observed dependencies examination, additionally the penetration ability of few analogues, not used as a hair dyes, had been studied; for those structures there are no INCI name in Figure 2).

The penetration ability analysis for selected dyes were carried out in model system: in side-by-side cells with liquid-crystalline lipid membranes. The lipids mixture corresponds to stratum corneum composition (ingredients by INCI name: Hydrogenated Lecithin, Cholesterol, Ceramide 3, Ceramide 2, Palmitic Acid, Oleic Acid).

Model system had been used previously for the estimation of penetration ability of biologically active agents, cosmetic ingredients, etc. It is well enough described in literature (Matsuzaki et al., 1993; Arct et al., 2002).

Dyes penetration ability was expressed as penetration coefficient – Kp [cm/h]. The results for nitrophenylenediamines are presented in Figure 3, for nitroaminophenols in Figure 4. Dyes on both graphs are placed in order with the lipophilicity (expressed as partition coefficient in octanol-water system – logPo/w) decrease.

No correlation between penetration ability and lipophilicity of investigated compounds has been observed. The diffusive properties could have a major impact on its penetration ability.

There are H-bond donors and acceptors present in analysed dyes structures. Attendance of these groups in dyes structures influence on its vehiculum-membrane partition coefficients and also cause its strong interactions with membrane liquid-crystalline structures, resulting in the decrease in diffusion ability.

The decrease in penetration ability with the N-hydroxyethyl substitution in nitrophenylenediamines structures has been observed. The effect is related to the increase in molecular size (the incorporation of some spatial obstacle cause worsening of the diffusion capacity inside the membrane), and also with the changes in membrane interactions ability (H-bonding). A sum of these effects is the reason of decrease in penetration ability.

A special interest has been put forward to those investigated dyes that exhibit intramolecular H-bonding capacity. Some differences in penetration have been observed for isomeric structures of nitrophenylenediamines (1a and 3a). The dye with strong intramolecular H-bonding capacity is able to limit the membrane interactions, and as that penetrates slightly more effectively.

Similar can be considered for isomeric nitroaminophenols (4a and 5a). The possibility to interact intramolecularly with higher bonding energy; additionally the “free” (not involved in intramolecular bonding) hydroxyl group occurrence (which interacts with membrane) in 4a structure cause its lower penetration ability, resulted from the diffusion limitations.
Fig. 2 Investigated hair dyes structures.
Fig. 3 Penetration of investigated nitrophenylenediamines form buffer solution (pH = 7.4).

Fig. 4 Penetration of investigated nitroaminophenols form buffer solution (pH = 7.4).
The results of presented studies showed, that the permeation of nitrophenylenediamines and nitroaminophenols used as hair dyes through the epidermal barriers depends significantly on their chemical structure. The introduction of substituents capable to form H-bondings can limit the permeation ability dramatically, even in case of lipophilic structures.

A further study are necessary, to distinguish the parameters which determinates hair dyes penetration ability (Quantitative-structure activity relationships), also further penetration analysis will allow to determine dependencies more precisely.

Acknowledgements
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References


8) Memorandum on hair dyes substances and their skin sensitising properties, Adopted by the SCCP during the 10th plenary of 19 December 2006, available at: http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_s_05.pdf


10) SCCP/1004/06: 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 8th plenary of 20 June, available at: http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_067.pdf2006


27) SCCP/1059/06: Opinion on 4-amino -3-nitrophenol Colipa n° B51, The SCCP adopted this opinion at its 11th plenary on 21 March 2007, available at:


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