OPINION OF THE SCIENTIFIC COMMITTEE ON COSMETIC PRODUCTS AND NON-FOOD PRODUCTS INTENDED FOR CONSUMERS

CONCERNING

# REQUEST FOR A RE-EVALUATION OF HAIR DYES LISTED IN ANNEX III TO DIRECTIVE 76/768/EEC ON COSMETIC PRODUCTS

adopted by the SCCNFP during the 23rd plenary meeting
of 18 March 2003

# 1. Terms of Reference

## 1.1 Context of the question

In the framework of the 26th Adaptation to technical progress of Directive 76/768/EEC on cosmetic products, 60 hair dyes have been listed in Annex III, part 2. Their listing in part 2 of Annex III implies that these substances are provisionally allowed until 30 September 2004 and that a re-evaluation has to be done before that date.

An ad-hoc Working Group of the Standing Committee on Cosmetic Products was set up which established a list of 46 hair dyes to be re-evaluated and formulated the questions to the Scientific Committee on Cosmetic Products and Non-food Products (SCCNFP) on each of these substances (Annex I of this document).

Furthermore the European Commission has received a letter from a Member State with data demonstrating the possible risk of mutagenicity/carcinogenicity of m-Phenylenediamine, COLIPA A3 (classified mutagen category 3, according to Directive 67/548/EEC).

## 1.2 Request to the SCCNFP

The SCCNFP was requested to answer the questions asked on 46 hair dyes (Annex I of this document) and on A3 (annex II of this document).

# 2. Opinion

The epidemiological data on the possible link between the use of hair dyes and bladder cancer strengthens the case further for a robust genotoxicity/carcinogenicity data package. The SCCNFP has therefore adopted the following papers that set the basic requirements to carry out a modern risk assessment :

- the assessment strategies for hair dyes, doc. n° SCCNFP/0553/02 of 17 December 2002;
- the proposal for a strategy for testing hair dye cosmetic ingredients for their potential of genotoxicity/mutagenicity, doc. n° SCCNFP/0566/02 of 4 June 2002;
- the opinion on the use of permanent hair dyes and bladder cancer risk, doc. n° SCCNFP/0484/01 of 12 June 2001

As a consequence, and in response to the above mandate, the SCCNFP is of the opinion that the previously submitted data for the above-mentioned 46 substances as well as for other existing substances listed in Annex III of Directive 76/768/EEC, like A3, no longer comply to present standards. A re-evaluation of these data could allow an incorrect conclusion with regard to the risks associated with the substances and could therefore be counter productive.

In order to prioritise safety evaluation, the SCCNFP recommends that lists of oxidative (permanent) hair dyes and semi-permanent hair dyes, together with their relevant oxidative agents and couplers, should be available, appropriately updated and reflect production volumes.

Therefore, and before any further consideration on the 46 substances mentioned in the mandate and on A3, the following information is required :

1. Complete information on the physico-chemical properties and the test protocols.
Production methods and full characterisation on purity and impurities in commercial and test batches should be included together with documentation for the reliability of the analytical methods used.
Information on the stability of the active substances in dye formulations and their degradation products (see Notes of Guidance, regularly updated by the SCCNFP, doc. n° SCCNFP/0321/00).

2. Data on genotoxicity/mutagenicity and on carcinogenicity following the SCCNFP-opinion "Proposal for a Strategy for Testing Hair Dye Cosmetic Ingredients for their Potential of Genotoxicity / Mutagenicity", doc. n° SCCNFP/0566/02 of 4 June 2002, and in accordance with the Notes of Guidance, regularly updated by the SCCNFP (doc. n° SCCNFP/0321/00).

3. Data on the percutaneous absorption following the requirements described in the Notes of Guidance, regularly updated by the SCCNFP.

Respective submissions should be presented for each substance and must include an evaluation by the applicant of the data.

Moreover, the Committee is aware that hair dyes in addition to those listed in the Annex of this opinion are currently available. Therefore, the SCCNFP recommends to the Commission that identical data packages should be provided for evaluation (as already stated in the opinion on A7, doc. n° SCCNFP/0129/99).

# Annex I - Questions on 46 hair dyes

## A 1 1,7-Naphthalenediol

It is not clear if the performed *in vivo* micronucleus tests are relevant because data on the cytotoxicity in the bone marrow are not mentioned (PCE/NCE ration). If the substance is not reaching the bone marrow a negative test can be false negative. Especially if the CA test *in vitro* is positive, the compound cannot be considered as non-mutagenic. Would the SCCNFP re-evaluate the in-vivo micronucleus test?
This substance gave a clear positive result in one of the *in vitro* mutagenicity assays. It induced chromosome damage in the metaphase analysis. The only *in vivo* data relates to the bone marrow, and furthermore this test, done in 1980, was not to current standards, or indeed to the 1983 OECD guidelines. Does the SCCNFP agree that data from an *in vivo* bone marrow micronucleus test and from an *in vivo* liver UDS assay are needed?
Does the SCCNFP agree that the MOS should be re-calculated using the NOEL of 10 instead of the applied NOEL of 50?

## A 14 o-Aminophenol

Taking into account the classification of the substance as a mutagen cat. 3 according to the Classification and Labelling Directive 67/548/EEC, would the SCCNFP explain why this substance can be safely used in cosmetic products?
Could the SCCNFP evaluate the background document provided by Norway?

## A 18 1,5-Naphthalenediol

It is not clear if the performed *in vivo* micronucleus tests are relevant because data on the cytotoxicity in the bone marrow are not mentioned (PCE/NCE ration). If the substance is not reaching the bone marrow a negative test can be false negative. Especially if the CA test *in vitro* is positive, the compound cannot be considered as non-mutagenic. Would the SCCNFP re-evaluate the in-vivo micronucleus test.
When considered by the SCC in 1991 the committee noted the inadequate data package – only one *in vitro* assay in Salmonella and an *in vivo* assay for clastogenicity in bone marrow using a dated protocol. Since the initial testing should be *in vitro* the SCC requested that an *in vitro* assay for chromosome damage be performed. This has been done and is clearly positive (as admitted by the SCCNFP) – but they now say it can be discounted because of the negative *in vivo* assay – despite the fact that it is not to current standards, and no data are available from a second tissue!
Does the SCCNFP agree that further data are needed from an *in vivo* bone marrow assay for clastogenicity to modern standards and from a second tissue?

## A 19 2,7-Naphthalenediol

It is not clear if the performed *in vivo* micronucleus tests are relevant because data on the cytotoxicity in the bone marrow are not mentioned (PCE/NCE ration). If the substance is not reaching the bone marrow a negative test can be false negative. Especially if the CA test *in vitro*

is positive, the compound cannot be considered as non-mutagenic. Would the SCCNFP re-evaluate the in-vivo micronucleus test?

### A 22 p-Methylaminophenol

Could SCCNFP set the specific concentration limits of impurities of p-aminophenol or other impurities, or does SCCNFP advise to use the general concentration limits laid down in the Commission Directive 67/548/EEC?
p-aminophenol is the main impurity (<2,5%) in this hair dye. p-aminophenol is classified as mutagenic cat. 3 (according to Directive 67/548/EEC relating to the classification, packaging and labelling of dangerous substances). Should an ingredient be banned on evidence of mutagenicity of an impurity or should a limit be set for the impurity?
Could the SCCNFP re-evaluate if nitrosamines can be formed when using this hair dye, and does it have any impact on the maximum authorised concentrations in the finished product?

### A 25 Hydroxybenzomorpholine

Could the SCCNFP re-evaluate if nitrosamines can be formed when using this hair dye, and does it have any impact on the maximum authorised concentrations in the finished product?

### A 27 4-Amino-2-hydroxytoluene

Although conflicting data were reported in a number of Salmonella assays, in 2 cases, using the most comprehensive range of bacteria, clear positive results were obtained. It should be assumed that this compound is positive in the Salmonella assay and has mutagenic potential. The only *in vivo* data are from a bone marrow micronucleous test done in 1976 that is not to current standards. Does the SCCNFP agree that data from an *in vivo* bone marrow micronucleus assay, and from a 2nd tissue (e.g. from an *in vivo* liver UDS assay) are needed before the positive *in vitro* can be discounted?
Could the SCCNFP give a clarification of the mutagenicity studies of this substance both from the old and new studies?

### A 28 3,4-Diamino-benzoic acid

It is not clear if the performed *in vivo* micronucleus tests are relevant because data on the cytotoxicity in the bone marrow are not mentioned (PCE/NCE ration). If the substance is not reaching the bone marrow a negative test can be false negative. Especially if the CA test *in vitro* is positive, the compound cannot be considered as non-mutagenic. Would the SCCNFP re-evaluate the in-vivo micronucleus test.

### A 31 2-Methyl-5-hydroxyethylaminophenol

Does SCCNFP consider a concentration of 2 % to be safe for use in cosmetic products?
In light of the sensitising potential of this substance, is there any human data, which justifies a use of a concentration of 2 %.

**A 42 2,4-Diamino-phenoxyethanol**

What is the reason for using the NOAEL of 56 mg/kg/day instead of using 21 mg/kg/day (from the subchronic 104 days study)?
The SCCNFP noted that the animal test showed moderate potential for allergy (30% response in a guinea pig model using adjuvant). Does the SCCNFP recommend a risk phrase?

**A 43 3-Amino-2,4-dichlorophenol**

New studies are needed on mutagenicity (*in vitro*, *in vivo*), subchronic studies and teratogenicity studies. Does the SCCNFP agree?
The only meaningful mutagenicity data is from a negative *in vitro* assay in Salmonella typhimurium. There is an inadequate *in vivo* bone marrow assay, carried out in 1980, which the SCCNFP recognised was not to current standards. In addition, the initial screening for mutagenic potential should be carried out using *in vitro* tests, which are more sensitive. Would the SCCNFP recommend that an *in vitro* metaphase analysis and a mouse lymphoma assay should be performed?
On the studies performed – what is the purity of the test substances s.b and s.c?

**A 44 2-Methylresorcinol**

In the teratogenicity study in rats, regarded as inadequate, slight increase (not statistically significant) was observed in the mean post-implantation loss with a corresponding decrease in the mean number of viable foetuses and implantation sites. The request for a teratogenicity study is supported.

**A 84 2-Amino-4-hydroxyethylaminoanisole**

Has the SCCNFP considered the short exposure time when calculating the bioavailability?

**A 97 6-Amino-o-cresol**

Can the SCCNFP justify scientifically how a classification of Class. A has been reached, taking into account the carcinogenicity data and the low MOS, and apart from the fact, that the hair dye is used only once a month?

**A 98 Hydroxyethyl-3,4-methylenedioxyaniline**

Can the SCCNFP justify scientifically how an agreement on a classification of Class A has been reached despite the low MOS and apart from the fact that the hair dye is used only once a month?
This substance gave positive results in the animal model and the SCCNFP recognised that this compound has sensitising potential. Would the SCCNFP recommend a risk phrase?

**A 112 2-Aminomethyl-p-amino-phenol**

It is not clear if the performed *in vivo* micronucleus tests are relevant because data on the cytotoxicity in the bone marrow are not mentioned (PCE/NCE ration). If the substance is not reaching the bone marrow a negative test can be false negative. Especially if the CA test *in vitro* is positive, the compound cannot be considered as non-mutagenic. Would the SCCNFP re-evaluate the in-vivo micronucleus test?
The animal studies on the sensitising potential of this hair dye are mostly inadequate but one study was judged by the SCCNFP as indicating sensitising potential. For consistency with other compounds would the SCCNFP recommend a warning phrase?

**A 113 2,4-Diamino-5-methyl-phenetol**

What is the chemical identity and the chemical purity of the test substance coded s.c?
Does the purity make a re-evaluation necessary?

**A 116 2,4-Diamino-5-methyl-phenoxyethanol**

It is not clear if the performed *in vivo* micronucleus tests are relevant because data on the cytotoxicity in the bone marrow are not mentioned (PCE/NCE ration). If the substance is not reaching the bone marrow a negative test can be false negative. Especially if the CA test *in vitro* is positive, the compound cannot be considered as non-mutagenic. Would the SCCNFP re-evaluate the in-vivo micronucleus test?

**A 118 Hydroxyethylaminomethyl-p-aminophenol**

It is not clear if the performed *in vivo* micronucleus tests are relevant because data on the cytotoxicity in the bone marrow are not mentioned (PCE/NCE ration). If the substance is not reaching the bone marrow a negative test can be false negative. Especially if the CA test *in vitro* is positive, the compound cannot be considered as non-mutagenic. Would the SCCNFP re-evaluate the in-vivo micronucleus test?
This substance gave positive results in the animal model for sensitising potential and the SCCNFP has recognised that this compound has sensitising potential. Would the SCCNFP recommend a risk phrase?

**A 121 Hydroxypropylbis (N-hydroxyethyl-p-phenylenediamine)**

It is not clear if the performed *in vivo* micronucleus tests are relevant because data on the cytotoxicity in the bone marrow are not mentioned (PCE/NCE ration). If the substance is not reaching the bone marrow a negative test can be false negative. Especially if the CA test *in vitro* is positive, the compound cannot be considered as non-mutagenic. Would the SCCNFP re-evaluate the in-vivo micronucleus test?
Could the SCCNFP explain the result of the positive chromosome aberration test and also explain why the result has been ignored?

**B 25 2-Nitro-p-phenylenediamine**

Would the SCCNFP re-evaluate their opinion on this substance mostly with regard to the genotoxicity and carcinogenicity data and their recent opinion on hair dyes and bladder cancer?

**B 31 HC Red No.13**

Which was the substance that was tested?
Will it be necessary to set purity criteria for the hair dye or is it the commercial product, which has obtained the Class? 1 from SCCNFP?

**B 37 HC Blue No.2**

It is not clear if the performed *in vivo* micronucleus tests are relevant because data on the cytotoxicity in the bone marrow are not mentioned (PCE/NCE ration). If the substance is not reaching the bone marrow a negative test can be false negative. Especially if the CA test *in vitro* is positive, the compound cannot be considered as non-mutagenic. Would the SCCNFP re-evaluate the in-vivo micronucleus test?
An uncommon tumor (mixed mesenchymal neoplasms of the kidney) was noted in female rats (2/50 of high dose group) and a marginal positive trend in the incidence of lymphomas in male mice. Does the SCCNFP agree that a carcinogenic risk, caused by the substance or by impurities present in the substance, can not be excluded?
Is there a need to set a purity grade of this compound when used in cosmetic products?

**B 49 2-Chloro-5-nitro-N-hydroxyethyl-p-phenylenediamine**

What is the mutagenic potential of the substance presented in 4% in the mixture?
Does SCCNFP agree that it would be necessary to set purity criteria for this hair dye?
What is the test used for the evaluation of the clastogenic potential *in vivo*?

**B 51 4-Amino-3-nitrophenol**

Since the micronucleus test was only performed on 2 mice and the protocol inadequate, does SCCNFP consider it necessary to perform a new micronucleus test?

**B 55 2-Amino-3-nitrophenol**

It is not clear if the performed *in vivo* micronucleus tests are relevant because data on the cytotoxicity in the bone marrow are not mentioned (PCE/NCE ration). If the substance is not reaching the bone marrow a negative test can be false negative. Especially if the CA test *in vitro* is positive, the compound cannot be considered as non-mutagenic. Would the SCCNFP re-evaluate the in-vivo micronucleus test?

**B 58 3-Methylamino-4-nitro-phenoxyethanol**

Could the SCCNFP re-evaluate if the substance is an eye irritant at the proposed concentration?

**B 66 HC Violet No.1**

It is not clear if the performed *in vivo* micronucleus tests are relevant because data on the cytotoxicity in the bone marrow are not mentioned (PCE/NCE ration). If the substance is not reaching the bone marrow a negative test can be false negative. Especially if the CA test *in vitro* is positive, the compound cannot be considered as non-mutagenic. Would the SCCNFP re-evaluate the in-vivo micronucleus test.

**B 67 HC Orange No.2**

The compound seems to be positive in one of the animal tests for sensitisation – does the SCCNFP recommend the use of a risk phrase ?

**B 71 HC Red No.10 + HC Red No.11**

Is there a need to set a purity grade of this compound when used in cosmetic products?
It is not clear if the performed *in vivo* micronucleus tests are relevant because data on the cytotoxicity in the bone marrow are not mentioned (PCE/NCE ration). If the substance is not reaching the bone marrow a negative test can be false negative. Especially if the CA test *in vitro* is positive, the compound cannot be considered as non-mutagenic. Would the SCCNFP re-evaluate the in-vivo micronucleus test?

**B 73 HC Blue No.12**

What about the 3 % of substance, which persist in the skin, does it have any influence on the calculation of MOS?
Could SCCNFP reconsider the MOS taking into account the pool of substance, which persists in the skin?

**B 74 HC Yellow No.6**

Can the SCCNFP justify scientifically (which means not that the dye is only used once a month) how an agreement on a classification of Class A has been reached despite the low MOS and apart from the fact that the hair dye is used only once a month.
Is there a need to set a purity grade of this compound when used in cosmetic products?
It has been stated in the teratogenicity study on rats that “the dose related decrease in sex ratio (% of male foetuses) is thought to be of no importance because no difference in total number of foetuses was seen”. Should more details on this issue be provided as changes in the sex ratio of foetuses may indicate a hormonal disruptive effect? Additionally, does the SCCNFP agree that minor anomalies observed in the lowest dose group might justify a request for further data?

**B 75 Hydroxyethyl-2-nitro-p-toluidine**

It is not clear if the performed *in vivo* micronucleus tests are relevant because data on the cytotoxicity in the bone marrow are not mentioned (PCE/NCE ration). If the substance is not reaching the bone marrow a negative test can be false negative. Especially if the CA test *in vitro* is positive, the compound cannot be considered as non-mutagenic. Would the SCCNFP re-evaluate the in-vivo micronucleus test?

**B 76 HC Yellow No. 12**

Is there a need to set a purity grade of this compound when used in cosmetic products?

**B 78 HC Blue No.10**

It is not clear if the performed *in vivo* micronucleus tests are relevant because data on the cytotoxicity in the bone marrow are not mentioned (PCE/NCE ration). If the substance is not reaching the bone marrow a negative test can be false negative (No CA test has been performed). Would the SCCNFP re-evaluate the in-vivo micronucleus test?

**B 82 HC Blue No. 9**

It is not clear if the performed *in vivo* micronucleus tests are relevant because data on the cytotoxicity in the bone marrow are not mentioned (PCE/NCE ration). If the substance is not reaching the bone marrow a negative test can be false negative. Especially if the CA test *in vitro* is positive, the compound cannot be considered as non-mutagenic. Would the SCCNFP re-evaluate the in-vivo micronucleus test?

**B 90 Hydroxyethyl-2,6-dinitro-p-anisidine**

It is not clear if the performed *in vivo* micronucleus tests are relevant because data on the cytotoxicity in the bone marrow are not mentioned (PCE/NCE ration). If the substance is not reaching the bone marrow a negative test can be false negative. Especially if the CA test *in vitro* is positive, the compound cannot be considered as non-mutagenic. Would the SCCNFP re-evaluate the in-vivo micronucleus test?
The result was positive in Salmonella typhimurium TA97 in the presence of rat S-9 but not in the absence of S-9. In addition it was positive in TA100 both in the presence and absence of S-9. It was negative in the nitroreductase deficient strains TA98-NR and TA100-NR both in the presence of rat S-9. The SCCNFP appears to have discounted the positive data because it was due to the high nitroreductase levels. However, this ignores the fact that the compound was positive in TA97 only in the presence of rat S-9 indicating that it is the rat liver enzymes that are activating the compound in this strain. Also nitroreductase activation may occur in humans. The *in vitro* mutagenicity data indicate that this compound has mutagenic potential. Does the SCCNFP agree that *in vivo* assays in 2 tissues are needed before concerns about these facts can be discounted?
Data are available from an *in vivo* bone marrow micronucleus test, but this has limitations. The compound has low toxicity and would have thought to be tested at 2 g/kg (the maximum level

recommended in the guidelines). The only dose used was 1.5 gram/kg and there was no indication that the compound reached the bone marrow. It is preferable that this study is repeated at higher dose levels. In addition, data are needed from a second tissue (preferably a liver UDS assay.

**B 92 6-Nitro-2,5-pyridinediamine**

It is not clear if the performed *in vivo* micronucleus tests are relevant because data on the cytotoxicity in the bone marrow are not mentioned (PCE/NCE ration). If the substance is not reaching the bone marrow a negative test can be false negative. Especially if the CA test *in vitro* is positive, the compound cannot be considered as non-mutagenic. Would the SCCNFP re-evaluate the in-vivo micronucleus test.
In view of the data from an inadequate bone marrow assay, data from an assay in a 2nd tissue (in-vivo liver UDS assay) should be requested.
Severe toxicological effects on brain, thyroid and testicles. The NOAEL of 5 mg/kg bodyweight in an ordinary 90 days test is not sufficient. Because of the severe effects more specialised toxicological tests for neurotoxicity and male reproduction is needed. Does the SCCNFP agree to that?
Due to the teratogenicity data a re-evaluation of the NOAEL is requested.

**B 98 HC Violet No. 2**

Does SCCNFP consider this substance to be a sensitiser according to the Directive 67/548/EEC?

**B 99 2-Amino-6-chloro-4-nitrophenol**

It is not clear if the performed *in vivo* micronucleus tests are relevant because data on the cytotoxicity in the bone marrow are not mentioned (PCE/NCE ration). If the substance is not reaching the bone marrow a negative test can be false negative. Especially if the CA test *in vitro* is positive, the compound cannot be considered as non-mutagenic. Would the SCCNFP re-evaluate the in-vivo micronucleus test?

**B 100 4-Hydroxy-propylamino-3-nitrophenol**

Is it necessary to take into account the cumulative effect of the consumers dyeing their hair with both oxidising- and non-oxidising colouring agents and that the MOS is low?

**C 12 Ponceau SX**

Very old assessment. Does the SCCNFP agree that a new evaluation should be requested?

**C 22 Acid Red 33**

Very old assessment. Does the SCCNFP agree that a new evaluation should be requested?

**C 43 Basic Violet 14**

Very old assessment. Does the SCCNFP agree that a new evaluation should be requested?

**C 46 Basic Blue 7**

Is the bioavailability of the substance when administered by oral route, taken into account when setting the NOEL? What is the purity grade of the substance called s.b.? Does the purity imply a re-evaluation of the hair dye?

**C50 Basic Blue 26**

Very old assessment. Does the SCCNFP agree that a new evaluation should be requested?

## Annex II – Question on A3

1. Is m-phenylenediamine safe when used as an oxidising colouring agent for hair dyeing at the authorised maximum concentration taking into account the data provided?

2. And/or does SCCNFP recommend further restrictions when it is used as an oxidising colouring agent for hair dyeing in cosmetic products ?