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

PROPOSAL

FOR

A STRATEGY FOR TESTING HAIR DYE COSMETIC INGREDIENTS FOR THEIR POTENTIAL GENOTOXICITY/MUTAGENICITY

adopted by the SCCNFP during the 20th plenary meeting
of 4 June 2002

1. Objective

Within the framework of hazard and risk assessment presented by hair dye ingredients, it is necessary to identify genotoxic/mutagenic chemicals and to evaluate the potential adverse health effects they may represent for the consumers.

The justification for such attention derives from the recent finding of a possible correlation between the use of hair dyes and the occurrence of bladder cancer (Opinion SCCNFP/0484/01 adopted during the 17th P1enary Meeting of 12th June 2001). (1)

A discussion paper regarding the "Assessment Strategies for Hair Dyes", was recently adopted during the 19th Plenary Meeting on 27th February 2002 (Opinion SCCNFP/0553/02). It states that in the context of a risk assessment of hair dyes the data on genotoxicity and mutagenicity must have been generated by studies performed under conditions, which conform to internationally accepted guidelines (OECD, EU) and to modern testing strategies. Moreover, the discussion paper also states that "according to the intended use, the dyes/dye-precursors must be tested alone and/or in combination with other substances to simulate the conditions of use". (2)

2. Hair Dyes

Hair dyeing formulations belong to 3 categories, i.e. for temporary, semi-permanent and permanent colouring of hair. The products for temporary dyeing of hair generally comprise of water soluble acid dyes and water soluble pigments, which are deposited on the surface of the hair. These colours are removable by a single effective shampooing. The formulations for semi-permanent dyeing of hair contain for the most part simple derivatives of nitroanilines, nitrophenylenediamines and nitroaminophenols. These dyes penetrate into the cuticle and partially into the cortex of the hair. As a result, the colouring effects of these dyes can resist 5-10 shampooing. The formulations for permanent hair colouring are marketed as two component kits. One component contains the dye precursors (such as *p*-phenylene- diamine, 2,5-diaminotoluene, N,N-bis(2-hydroxymethyl)-p-phenylene diamine, p-aminophenol etc.) and couplers (such as resorcinol, chlororesorcinol, methyl resorcinol, α-naphthol, m-aminophenol, m-phenylenediamine, etc.) in an alkaline soap or syndet base, and the other component is a stabilised solution of hydrogen peroxide. The two components are mixed immediately prior to use. The precursors and peroxide diffuse into the hair shaft, where colour formation takes place after a cascade of chemical reactions. The dye precursors are oxidised by hydrogen peroxide to p-benzoquinone imines/diimines, which are reactive intermediates in the colour formation. The couplers, which are relatively stable to hydrogen peroxide, undergo rapid reaction with the intermediates resulting in dinuclear, trinuclear or polynuclear colour molecules. These molecules are too large to escape from hair structure. These dyes are also called oxidative hair dyes. Hydrogen peroxide in the oxidative hair dye formulations also serves as bleaching agent for the natural pigment of the hair. The colour formation (shades) is dependent on precursors present in the dyeing solution, its pH and the time of contact with the hair (3,10,11).

Oxidative hair dyes are resistant to fading by shampooing, but re-colouring of hair is required every 6 weeks approximately, due to hair growth. The time of contact of the dyeing solution with the hair scalp may vary from 15-45 minutes. Oxidative hair dyes represent the major segment of the hair colouring market.

The assessment of genotoxicity/mutagenicity potential of oxidative hair dyes is rather complex, because it involves dye precursors, intermediates, reaction and final product(s) at the same time. The hazard of the oxidative hair dyes must therefore be evaluated by testing the individual ingredients as well as by testing a combination of relevant ingredients, so that the genotoxic/mutagenic potential of the novel reaction products formed during the application period could be evaluated.

3. Mutagenicity/Genotoxicity

3.1. Preamble

1. Genotoxic properties may be defined as the potential of a chemical to react with DNA. Under certain circumstances (when lesions are not repaired or misrepaired...) the induced lesions may be converted into mutations. Tests for detecting genotoxic potentials comprise :

a. DNA adduct determination;
b. Sister Chromatid Exchanges (SCEs)
c. Mitotic recombinations
d. Detection of unscheduled DNA synthesis that is indicative of nucleotide excision repair.

2. Mutation is defined as a permanent change of the amount or structure in the genetic material of an organism. These changes may result in heritable changes of the organism. These alterations may involve :

a. genes, block of genes and/or
b. whole chromosomes (structural and/or numerical)

3. During the risk assessment process of chemicals it is then necessary to pay attention to both genotoxicity and mutagenicity tests. Moreover, in addition to the detection of intrinsic genotoxic potentials of chemicals, the extent of human exposure should drive the need for an additional battery of tests.

The second value of the mutagenicity/genotoxicity testing also lies in its ability to identify chemicals that may, under certain exposure conditions, either cause cancer by a predominantly genotoxic mechanism or induce the initial phase of carcinogenic processes. (5)

3.2. Testing Strategy

Adequate knowledge of the formulation of the oxidative hair dye must be taken into consideration before genotoxic/mutagenic testing is performed. Moreover, genotoxic/mutagenic testing of the mixture of precursor(s), coupler(s) and oxidant, to which the consumer is exposed, should be performed using several *in vitro* test methods.

There may be instances where alternative tests to those specified below are more appropriate: in such cases a scientific justification must be presented.

Stage 1: *In vitro* tests

1. Bacterial Reverse Mutation Test (OECD 471, 21st July 1997; EC B.13/14, 19th May 2000) (5, 12)

2. Test for clastogenicity and for indications of aneugenicity :

   a. (preferred test) *In vitro* Mammalian Chromosome Aberration Test (OECD 473, 21st July 1977; EC B.10 19th May 2000)(5, 12)

   or

   b. *In vitro* Micronucleus Test* (not yet adopted by OECD)

3. *In vitro* Mammalian Cell Gene Mutation Test (currently, the preferred choice is the mouse lymphoma assay; the CHO HGPRT assay has more shortcomings)(OECD 476 21st July 1997; EC B.17, 19th May 2000) (5, 12)

4. Unscheduled DNA Synthesis in Mammalian Cells *in vitro* (OECD 482, 23rd Oct. 1986; EC B.18, 18th November 1987) (5, 13)

The need to apply four *in vitro* tests for evaluating the genotoxic/mutagenic potential of hair dyes (precursors or combinations of different chemicals) has three justifications :

1. *In vitro* tests are particularly useful for gaining an understanding of the potential mutagenicity/genotoxicity of a substance and they have a critical role in the testing strategy, as well as in the choice of appropriate animal tests for eventual future investigations;
2. Several aromatic amines are not mutagenic in bacterial mutagenicity tests, whereas they may be identified as mutagens in a mammalian cell gene mutation test (4);
3. Two tests for gene mutation (bacterial and mammalian cell assays) are required when human exposure is expected to be extensive, as in the case of hair dyeing (6);

All mutagenicity/genotoxicity studies should be carried out under the most rigorous protocols, such as those adopted by OECD or EC. (5, 7).

Interpretation of results

1. If **NEGATIVE** results are obtained in Stage 1 screening tests, the substance or the combinations of the precursors (the intermediate and the final products) can be

* When there are indications that compound has aneugenic properties, the use of appropriate staining procedures is required.

considered "not mutagenic" i.e. showing no evidence of intrinsic mutagenicity/genotoxicity.

2. If **POSITIVE** results are obtained in one or more Stage 1 screening tests, the substance or the combinations of the precursors (the intermediate and the final products) must be considered "*in vitro* mutagenic", i.e. showing evidence of intrinsic mutagenicity/genotoxicity.

   Further investigation using *in vivo* assays is strongly indicated. (8)

   Substances or combinations of the precursors (the intermediate and the final products) showing pronounced responses in several in vitro assays should be considered "mutagenic", if no relevant in vivo data are available.

3. If **EQUIVOCAL** results are obtained, further *in vitro* testing employing different cellular systems with the same endpoint should be considered.

   If this does not resolve the question then *in vivo* testing (Stage 2) is required.

Stage 2: *in vivo* tests

The choice of *in vivo* tests of Stage 2 should be justified by the results obtained with Stage 1 testing.
Before undertaking *in vivo* studies, an in-depth review of *in vitro* results is necessary to assess the biological significance of the *in vitro* effects obtained at different endpoints (e.g. lack of absorption, inability of active metabolite to reach DNA, rapid detoxification and elimination of reaction products, existence of confounding factors such as pH, osmolarity, cytotoxic or cytostatic effects). The following tests are those commonly preferred:

- Mammalian Erythrocyte Micronucleus Test (OECD 474, 21st July 1997; EC B.12, 19th May 2000) (5, 12)*

- Mammalian Bone Marrow Chromosome Aberration Test (OECD 475, 21st July 1997; EC B.11, 19th May 2000) (5, 12)

- Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *in vivo* (OECD 486, 21st July 1997; EC B.39, 19th May 2000) (5, 12)

Interpretation of results

1. If **NEGATIVE** results are obtained with Stage 2 tests according to OECD criteria, the substance or the combinations of the precursors (the intermediate and the final products) can be considered "not an *in vivo* somatic cell mutagen".

* If aneugenicity is suspected or demonstrated in Stage 1, the use of kinetochore or centromeric staining procedures is necessary

2. If **POSITIVE** results are obtained in any of the Stage 2 tests, the substance, or the combinations of the precursors (the intermediate and the final products) must be considered "mutagenic in somatic cells of mammals (*in vivo*)".

3. If **EQUIVOCAL** results are obtained in different Stage 2 tests, it is necessary to review all available information and to consider whether further testing is justified or a conclusion can be reached based on the weight of evidence.

4. Rationale

The SCCNFP "Notes of Guidance for Testing of Cosmetic Ingredients for Their Safety Evaluation" suggests that "bacterial reverse mutation test (or *in vitro* mammalian cell gene mutation test) and *in vitro* mammalian cell chromosome aberration test provide in general sufficient evidence of mutagenic/genotoxic potential. Use of *in vivo* tests is limited to confirmation of a mutagenic activity already observed or suspected *in vitro*."
Taking into account the toxicological profile of the substance and its chemical structure, further information by applying additional genotoxicity tests may be necessary.

The case of the hair dye as illustrated above justifies the strategy of additional testing presented in this opinion.

The requirement for the testing strategy for mutagenicity/genotoxicity potential of the hair dyes or combinations of reaction products is in line with the main current recommended Guidelines for Mutagenicity Testing adopted by EC for chemicals in other sectors involving Consumers' Exposure (7), such as Industrial Chemicals produced at a level of 10-100 T/year, Pharmaceuticals, Food additives, Packaging Materials, Plant Protection Products.
The UK Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment and the European Commission indicate the need of Stage 1 and 2 testing procedures similar to those presented in this opinion paper for all compounds with prolonged human exposure. (6,9)

5. Reference

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