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

THE SCCNFP'S NOTES OF GUIDANCE FOR THE TESTING OF COSMETIC INGREDIENTS AND THEIR SAFETY EVALUATION

5TH REVISION

Adopted by the SCCNFP during the25th plenary meeting
of 20 October 2003

Nam et ipsa scientia potestas est
For knowledge itself is power

Francis Bacon (1561- 1626) Essays

The "*Notes of Guidance for Testing of Cosmetic Ingredients and Their Safety Evaluation by the SCCNFP*" is a document compiled by the members of the Scientific Committee on Cosmetic Products and Non-Food Products intended for consumers (SCCNFP, replacing the former SCC). The document contains relevant information on the different aspects of testing and safety evaluation of cosmetic ingredients. It is designed to provide guidance to public authorities and cosmetic industry, in order to improve harmonized compliance with Directive 76/768/EEC[*] and in particular by the Sixth (Dir. 93/35/EEC[†]) and "Seventh" (Dir. 2003/15/EC[‡]) Amendments to this Directive.

The "Notes of Guidance" are regularly revised and updated in order to incorporate the progress of scientific knowledge in general, and the experience gained in particular, in the field of testing and safety evaluation of cosmetic ingredients.

The last revision took place in 2000 (SCCNFP/0321/00, Final[§]). Since then, several new opinions of importance to the content of this guidance document have been adopted and they form the basis of this new revision. In addition, the whole document has been reorganised and restructured in a more comprehensive way.

All the annexes have been incorporated in the body text, which now contains the full basic information. Individual SCCNFP opinions are not taken up in detail, but are briefly summarized and clearly referred to. They have become too numerous to be taken up in full length in one document.

The "Notes of Guidance" should not be seen as a checklist, but have been compiled to provide assistance in the complex process of testing and safety evaluation of cosmetic ingredients.
Input of scientists from industry and the European Cosmetic Toiletry and Perfumery Association (COLIPA), is gratefully acknowledged.

The Chairperson

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[*] **Council Directive 76/768/EEC** of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.
Official Journal L 262, 27/09/1976 p.169.

[†] **Council Directive 93/35/EEC** of 14 June 1993 amending for the sixth time Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products.
Official Journal L 151, 23/06/1993 p.32.

[‡] **Directive 2003/15/EC** of the European Parliament and of the Council of 27 February 2003 amending Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products.
Official Journal L66, 11/03/2003 p.26.

[§] **SCCNFP/0321/00, Final** : Notes of Guidance for Testing of Cosmetic Ingredients for Their Safety Evaluation, 4th revision *adopted by the SCCNFP during the plenary meeting of 24 October 2000.*

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ABBREVIATIONS AND GLOSSARY OF TERMS

3R	Refinement, Reduction, Replacement
3T3 NRU PT	3T3 Neutral Red Uptake Phototoxicity Test
Acceptability test	A test intended to confirm the fulfilment of the expectations for a cosmetic product in-use [SCCNFP/0068/98]
Alternative methods	All those procedures which can completely replace the need for animal experiments, which can reduce the number of animals required, or which can reduce the amount of pain and stress to which the animal is subjected in order to meet the essential needs of humans and other animals [Rogiers et al. 2000]
Art.	Article
BCOP	Bovine Corneal Opacity and Permeability
BSE	Bovine Spongiform Encephalopathy
BW	Body Weight
CAS	Chemical Abstracts Service
CI	Colour Index
CMR	Carcinogenic, Mutagenic, toxic to Reproduction
Colipa	European Cosmetic Toiletry and Perfumery Association
Compatibility test	A test intended to confirm that there are no harmful effects when applying a cosmetic product for the first time to the human skin or mucous membrane; the test must involve exposure (normal or slightly exaggerated) which closely mimics typical consumer use of the product [based on SCCNFP/0068/98]
Cosmetic ingredient	Any chemical substance or preparation of synthetic or natural origin, used in the formulation of cosmetic products. A cosmetic ingredient may be : 1- a chemically well-defined single substance with a molecular and structural formula, 2- a complex preparation, requiring a clear definition and often corresponding to a mixture of substances of unknown or variable composition and biological nature, 3- a mixture of 1 and 2, used in the formulation of a finished cosmetic product. [based on Art. 5a of 93/35/EEC and SCCNFP/0321/00])
Cosmetic product	Any substance or preparation intended to be placed in contact with the various parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odours and/or protecting them or keeping them in good condition [Art. 1 of 93/35/EEC]
CTFA	Cosmetic, Toiletry and Fragrance Association
DA_a*	Dermal Absorption reported as amount/cm²
DA_p*	Dermal Absorption expressed as a percentage

* used in the calculation of the Systemic Exposure Dosage (see section 3-7.3).

Dermal / percutaneous absorption	Amount of dermally applied substance remaining in the residual skin (excluding the stratum corneum) plus the amount of dermally applied substance which has transpassed the skin and is detected in the receptor fluid. The sum is considered to be systemically available (= dermal bioavailability) [based on OECD 2000, Diembeck et al., 1999, ECETOC 1993].
DG	Directorate-General
DG ENTR	Directorate-General Enterprise
DG ENV	Directorate-General Environment
DG SANCO	Directorate-General Health and Consumer Protection
Dir.	Directive
DNA	DeoxyriboNucleic Acid
Doc.	Document
Dosage	A general term comprising of dose, its frequency and duration [General Introduction: Part B, 96/54/EC]
Dose	The amount of test substance administered. Dose is expressed as weight (grams or milligrams) or as weight of test substance per unit of weight of test animal (e.g. milligrams per kilogram body weight), or per skin surface unit (e.g. milligrams per square centimetre of skin), or as constant dietary concentrations (parts per million or milligrams per kilogram of food) [based on General Introduction: Part B, 96/54/EC]
Dose-descriptor	The calculated amount of a test substance administered daily (e.g. mg/kg body weight/day) that in the case of a non-threshold carcinogen increases the net frequency of tumours at a specific site by a certain percentage (e.g. T_{25}) [Dybing et al. 1997]
EC	European Communities
ECB	European Chemicals Bureau
ECVAM	European Centre for the Validation of Alternative Methods
EEC	European Economic Commission
EINECS	European INventory of Existing commercial Chemical Substances
ELINCS	European List of Notified Chemical Substances
ESAC	ECVAM Scientific Advisory Committee
EST	Embryotoxic Stem cell Test
EU	European Union
F*	Frequency of application
Finished cosmetic product	The cosmetic product in its final formulation, as placed on the market and made available to the final consumer, or its prototype [2003/15/EC]
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GPMT	Guinea Pig Maximisation Test
HET-CAM	Hen's Egg Test-Chorio Allantoic Membrane
HPRT	Hypoxanthine-guanine PhosphoRibosyl Transferase

* used in the calculation of the Systemic Exposure Dosage (see section 3-7.3).

HPV	High Production Volume
IFRA	International Fragrance Research Association
***In vitro* test method**	Biological method : using organs, tissue sections and tissue cultures, isolated cells and their cultures, cell lines and subcellular fractions Non-biological method : such as computer modelling, chemical interaction studies, receptor binding studies, … [based on Rogiers et al. 2000]
***In vivo* test method**	Test method using living (experimental) animals [Rogiers et al. 2000]
INCI	International Nomenclature of Cosmetic Ingredients
INN	International Non-proprietary Name
IPCS	International Programme on Chemical Safety
IUPAC	International Union of Pure and Applied Chemistry
JRC	Joint Research Centre
LD_{50}	Median Lethal Dose 50% : a statistically derived single dose of a substance that can be expected to cause death in 50% of the dosed animals (expressed in mg/kg body weight) [General Introduction: Part B, 96/54/EC]
LLNA	Local Lymph Node Assay
LO(A)EL	Lowest Observed (Adverse) Effect Level : the lowest dose or exposure level within a specific test system, where (adverse) treatment-related findings are observed [ECB 2003]
MM	MicroMass
MoS	Margin of Safety
MR	Mitotic Recombination
MSDS	Material Safety Data Sheet
MTT	3-(4,5)-dimethyl-2-thiazolyl-2,5-dimethyl-2H-tetrazolium bromide
MW	Molecular Weight
NO(A)EL	No Observed (Adverse) Effect Level : the highest dose or exposure level within a specific test system, where no (adverse) treatment-related findings are observed [General Introduction: Part B, 96/54/EC]
NRU	Neutral Red Uptake
OECD	Organisation for Economic Co-operation and Development
Ph. Eur.	European Pharmacopoeia
PIR	Product Information Requirement
P_{ow}	n-octanol / water partition coefficient
ppm	parts per million (e.g. mg/kg)
Prototype	A first model or design that has not been produced in batches, and from which the finished cosmetic product is copied or finally developed [2003/15/EC]
QSAR	Quantitative Structure-Activity Relationship
RBC	Red Blood Cell
RIVM	RijksInstituut voor Volksgezondheid en Milieu
SC	Stratum Corneum

SC or dermal adsorption	(= substantivity) amount of topically applied test substance present in or sticking to the SC. It is considered not to be systemically available and is excluded from risk assessment [based on Diembeck et al. 1999]
SCC	Scientific Committee on Cosmetology
SCCNFP	Scientific Committee on Cosmetic products and Non-Food Products intended for consumers
SCE	Sister Chromatid Exchange
SED	Systemic Exposure Dose
SHE	Syrian Hamster Embryo
SI	Stimulation Index
SRM	Specified Risk Material
SSA*	Skin Surface Area
SSC	Scientific Steering Committee
Syndet	Synthetic detergent
TER	Transcutaneous Electrical Resistance
TEWL	TransEpidermal Water Loss
TIF	Technical Information File
Toxicodynamics	Cover the process of interaction of chemical substances with target sites and the subsequent reactions leading to adverse effects [ECB 2003]
Toxicokinetics	Describe the time-dependent fate of a substance within the body. They include absorption, distribution, biotransformation and/or excretion [ECB 2003]
TSE	Transmissible Spongiform Encephalopathy
UDS	Unscheduled DNA Synthesis
UV	UltraViolet (wavelengths UV-A : 315-400 nm, UV-B : 280-315 nm, UV-C : 100-280 nm) [2000/33/EC]
Valid method	A technique that has not necessarily gone through the complete validation process, but for which a sufficient amount of scientific data exist proving its relevance and reliability [Rogiers 2003]
Validated method	A method for which the relevance and reliability are established for a particular purpose according to the criteria established by ECVAM, taking into account that a prediction model needs to be present from the start of the validation procedure [based on Balls et al. 1997 and Worth et al. 2001]
VIS	VISible light (wavelength 400-800 nm)
WEC	Whole Embryo Culture
WHO	World Health Organisation

* used in the calculation of the Systemic Exposure Dosage (see section 3-7.3).

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2000/33/EC - Commission Directive 2000/33/EC of 25 April 2000 adapting to technical progress for the 27th time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.
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SCCNFP/0321/00, Final : Notes of Guidance for Testing of Cosmetic Ingredients for Their Safety Evaluation, 4th revision, *adopted by the SCCNFP during the plenary meeting of 24 October 2000*.

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1. INTRODUCTION

According to Article 1 of Council Directive 76/768/EEC and its amendments, *a **cosmetic product** shall mean any substance or preparation intended to be placed in contact with the various parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odours and/or protecting them or keeping them in good condition*.

Article 2 of that same Directive specifies that a cosmetic product *must not cause damage to human health when applied under normal or reasonably foreseeable conditions of use*.

Cosmetic products have a history, covering thousands of years, in using a variety of ingredients derived from plants, animals and mineral sources. Modern technology has added an important number of ingredients from synthetic and semi-synthetic origin.
Present-day use of cosmetic products has become very extensive and affects most population groups within the European Union, although the degree and nature may vary within the different Member States.

In practice, cosmetic products have rarely been associated with serious health hazards, which, however, does not mean that cosmetics are safe in use *per se*. Particular attention is needed for long-term safety aspects, since cosmetic products may be used extensively over a large part of the human lifespan. Therefore, the safety-in use of cosmetic products has been established in Europe by controlling the ingredients, their chemical structures, toxicity profiles, and exposure patterns [93/35/EEC*].

In June 1982 (Report EUR 8794), long before the Sixth Amendment to Dir. 76/768/EEC [93/35/EEC] was implemented, a pioneer document was issued by the former SCC dealing with "Guidelines for the toxicity testing of cosmetic ingredients.". Later, a number of documents followed that took into account both the experience gained by the SCC/SCCNFP in evaluating the toxicological profile of an important number of cosmetic ingredients and the development of the scientific knowledge, in particular in the field of toxicology.

At present, safety evaluation of cosmetic ingredients is carried out by the SCCNFP using data obtained from animal studies (*in vivo*), *in vitro* experiments, QSAR (quantitative structure activity relationship) calculations, clinical studies, epidemiological studies and accidents.

With the implementation of Dir. 2003/15/EC†, the need for appropriate *in vitro* tests for the safety evaluation of cosmetic ingredients and products becomes crucial.

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* **Council Directive 93/35/EEC** of 14 June 1993 amending for the sixth time Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products.
Official Journal L 151, 23/06/1993 p.32.

† **Directive 2003/15/EC** of the European Parliament and of the Council of 27 February 2003 amending Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products.
Official Journal L66, 11/03/2003 p.26.

The SCCNFP would like to stress that currently available *in vitro* methods only constitute a fraction of the alternative methodology meant and described by Russell et al [1959*], proposing the ultimate alternative methodology, namely replacement of the laboratory animal by non-sentient material (organs, tissue sections, cell cultures, …).
Nevertheless, although replacement remains the ultimate goal, reduction of the number of animals and refinement of the methodology by reducing the pain and distress of the animals, provide realistic and significant improvements of actual testing methods and strategies.

In the present update, the state-of-the-art with respect to the full 3R strategy (refinement, reduction and replacement) of Russell et al [1959], adopted by the European Commission, is incorporated.
In particular, the SCCNFP has given special attention to those alternative methods that are suitable for the safety testing of cosmetic ingredients. These are taken up in the appropriate chapters.

The revised "Notes of Guidance" are concerned with testing and safety evaluation of the cosmetic ingredients listed in Annexes III, IV, VI, and VII of Dir. 76/768/EEC and those for which safety concerns have been expressed, but are also of interest to all cosmetic ingredients intended to be incorporated in a finished cosmetic product.

Although the "Notes of Guidance" have not been particularly written for the latter purpose, they indeed can be of practical use in making a TIF (Technical Information File) or PIR (Product Information Requirement) for a finished cosmetic product as required by Dir. 93/35/EEC.

These "Notes of Guidance" should not be seen as a checklist. Attempts have been made to incorporate some standardised procedures, exposure patterns, formulation types, etc., but the safety evaluation of cosmetic ingredients and finished products remains a scientific exercise that can only be performed on a case-by-case basis.
When major deviations from standardised protocols / procedures in the safety evaluation process occur, a scientific justification is essential.

It is self-evident that this version of the "Notes of Guidance" will require further revision as scientific knowledge advances. As some topics in the cosmetic field are very dynamic and regularly discussed, it is proposed to perform this revision on a yearly basis.

* Russell B, Russell WMS, Burch RL.
The principles of Humane Experimental Technique.
Methuen and Co Ltd, London (reprinted by the Universities Federation for Animal Welfare UFAW, 1992, Potters Bar, Herts), UK, 1959.

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

2-1 HISTORICAL BACKGROUND

The Scientific Committee on Cosmetology (SCC) was established on 19 December 1977 by Commission Decision 78/45/EEC; the purpose was to assist the European Commission in examining the complex scientific and technical problems surrounding the drawing up and amendment of European Union (EU) rules governing the composition, manufacture, packaging, and labelling of cosmetic products marketed in EU countries. The Committee was to be renewed every three years.
In 1997 a restructured Scientific Committee, named Scientific Committee on Cosmetic products and Non-Food Products intended for consumers (SCCNFP), has been established by Commission Decision 97/579/EC. It is composed of independent scientists in the field of medicine, toxicology, pharmacy, dermatology, biology, chemistry, and other disciplines, collectively covering the widest possible range of expertise for this multidisciplinary committee.
Since 1997, the SCCNFP has adopted a series of Scientific Opinions related to the improvement of the safety evaluation of cosmetic ingredients [http://europa.eu.int/comm/food/fs/sc/sccp/outcome_en.html/]. These opinions are part of the revised "Notes of Guidance".

2-2 MANDATE

In accordance with Commission Decision 97/579/EC, the SCCNFP is competent to answer scientific and technical questions concerning consumer health relating to cosmetic products and non-food products intended for consumers, especially substances used in the preparation of these products, their composition and their use, as well as their types of packaging and labelling.
In addition, the Commission may request advice from the Committee on any other matter in the field of its competence, and moreover, upon its own initiative, the Committee may draw the attention of the Commission to potential or emerging hazards according to its field of competence.

The work of the SCCNFP can be divided in two main domains, namely matters related to cosmetic products and those related to non-food consumer products.
Where cosmetic products are concerned, the consultation of the SCCNFP is **compulsory** (Art.8.2 of the Cosmetic Products Directive [76/768/EEC]), whereas it is **not compulsory** in the domain of non-food products.

2-3 RULES OF PROCEDURE

The SCCNFP Rules of Procedure are laid down in SCCNFP/0042/98, adopted during the Plenary Meeting of March 2001.

2-4 OUTCOME OF DISCUSSIONS

The opinions adopted by the Scientific Committee on Cosmetology at the Commission's request were formerly included in EC-Reports (EUR 7297, 8634, 8794, 10305, 11080, 11139, 11303, 14208). From 1997 on, all opinions have been published on the Internet and can be accessed through http ://europa.eu.int/comm/food/fs/sc/sccp/outcome_en.html. They are listed chronologically according to the plenary meetings during which they have been adopted. **Therefore an SCCNFP opinion can easily be located on the Website through its adoption date.**
The opinions not only refer to cosmetic ingredients included in Annexes II, III, IV, VI and VII of Council Directive 76/768/EEC, but they also cover a broad range of diverging scientific issues related to the safety of cosmetic ingredients and finished products.

2-4.1 The "Notes of Guidance"

One of the main responsibilities of the former SCC and the present SCCNFP has been to recommend a set of guidelines to be taken into consideration by the cosmetic and raw material industry in developing adequate studies to be used in the safety evaluation of cosmetic ingredients. The SCC and its successor SCCNFP, have adopted, in this respect, the following opinions :

(a) Notes of Guidance for the toxicity testing of cosmetic ingredients (28 June 1982; EU Report 8794);
(b) Notes of Guidance for testing of cosmetic ingredients for their safety evaluation (SPC/803/5/90, First Revision);
(c) Notes of Guidance for testing of cosmetic ingredients for their safety evaluation (DGXXIV/1878/97, Second Revision);
(d) Notes of Guidance for testing of cosmetic ingredients for their safety evaluation (SCCNFP/0119/99, Third Revision).
(e) Notes of Guidance for testing of cosmetic ingredients for their safety evaluation (SCCNFP/0321/00, Fourth Revision).

The Notes of Guidance are regularly updated in order to incorporate new knowledge and scientific advances.

As cosmetic ingredients are chemical substances, these guidelines include the toxicological test procedures reported in Annex V to the Dangerous Substances Directive [67/548/EEC] and its adaptations to technical progress. They represent the basic toxicity testing procedures needed to evaluate different toxicological endpoints and are internationally accepted as being the result of long-term scientific agreement. The procedures to be followed for chemical substances include a large number of *in vivo* animal models and a limited number of studies based on *in vitro* models.
In addition, when evaluating the information given by industry on cosmetic ingredients meant to be incorporated in one of the Annexes of the Cosmetics Directive, the SCCNFP commonly accepted testing procedures in accordance with the OECD (Organisation for Economic Co-operation and Development) Guidelines, and well-documented scientifically justified methods based on an *in vitro* model or other 3R procedures.

The early acceptance by the SCCNFP of the *in vitro* study on dermal / percutaneous absorption using human/pig skin is an example of the pro-active work of the Committee. Before an OECD Guideline became available, *in vitro* dermal / percutaneous absorption results were accepted by the SCCNFP on the condition that the methods were scientifically well developed. For this reason, a set of guidelines for percutaneous absorption studies was established by the SCCNFP [SCCNFP/0167/99]. These have been reviewed in 2003 [SCCNFP/0750/03].

Over the years, several alternative methods have been further developed and 3 *in vitro* models for assessing skin corrosivity and phototoxicity have been incorporated in Annex V of Directive 67/548/EEC [2000/33/EC].

In view of the fact that in the cosmetic field the "Seventh Amendment" [2003/15/EC] imposes deadlines for banning animal testing, not only for finished cosmetic products, but also for their ingredients, much attention is given to the use of alternative methods in the safety evaluation of cosmetic ingredients and finished products throughout the whole "Notes of Guidance".

2-4.2 The status of cosmetic ingredients included in Annexes II, III, IV, VI and VII of Dir. 76/768/EEC

Since its establishment in 1997, the SCCNFP has provided opinions on more than 350 chemical substances and/or their mixtures. The majority of these opinions have been adopted into Cosmetic Legislation, more specifically they have been taken up in the Annexes to Dir. 76/768/EEC (Art. 8.2 and Art. 10 of Dir. 76/768/EEC), and have been used by the risk managers. The actual status of all annexes is shown below :

	STATUS SEPTEMBER 2003
Annex II (forbidden substances)	449 entries
Annex III, Part 1 (restrictions)	95 substances
Annex III, Part 2 (restrictions, provisionally allowed)	62 substances
Annex IV, Part 1 (list of colouring agents)	157 colourants
Annex IV, Part 2 (colouring agents, provisionally allowed)	empty
Annex VI, Part 1 (preservatives)	56 preservatives
Annex VI, Part 2 (preservatives, provisionally allowed)	empty
Annex VII, Part 1 (UV filters)	27 UV filters
Annex VII, Part 2 (UV filters, provisionally allowed)	empty

2-4.3 General issues taken up in the "Notes of Guidance"

In addition to the revision of the Notes of Guidance and the study of toxicological dossiers of cosmetic ingredients for inclusion in one of the Annexes of Dir. 76/768/EEC, some specific general issues have been addressed by the SCCNFP. Examples of these include :

a. Guidelines for human testing in cosmetic science

- Guidelines on the use of human volunteers in the testing of potentially cutaneous irritant cosmetic ingredients or mixtures of ingredients [SCCNFP/0003/98].
- Guidelines on the use of human volunteers in compatibility testing of finished cosmetic products [SCCNFP/0068/98].
- Opinion concerning the predictive testing of potentially cutaneous sensitising cosmetic ingredients or mixtures of ingredients [SCCNFP/0120/99].
- Opinion concerning basic criteria of the protocols for the skin compatibility testing of potentially cutaneous irritant cosmetic ingredients or mixtures of ingredients on human volunteers [SCCNFP/0245/99].

b. The use of alternative methods in the safety assessment of cosmetics

- Opinion on the use of alternative methods to animal testing in the safety evaluation of cosmetic ingredients or mixtures of ingredients [SCCNFP/0103/99].
- Memorandum concerning the actual status of alternative methods to the use of animals in the safety testing of cosmetic ingredients [SCCNFP/0546/02].

c. Bovine Spongiform Encephalopathology (BSE) issues related to cosmetic ingredients

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- Opinion concerning amendment to entry n° 419 of Annex II to Directive 76/768/EEC on Cosmetic Products [SCCNFP/0521/01].
- Opinion concerning amendment to entry n° 419 of Annex II to Directive 76/768/EEC on Cosmetic Products [SCCNFP/0552/02].
- Opinion concerning amendment to entry n° 419 of Annex II to Directive 76/768/EEC on Cosmetic Products [SCCNFP/0612/02].
- Opinion concerning use of specified risk materials in cosmetics : clarification for tallow derivatives [SSCNFP/0724/03].

d. CMR (Carcinogenic / Mutagenic / toxic to Reproduction) substances in cosmetics

- Opinion concerning chemical ingredients in cosmetic products classified as carcinogenic, mutagenic or toxic to reproduction according to the Chemicals Directive 67/548/EEC [SCCNFP/0474/01].

e. Hair dyes and their specific safety assessment

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- Opinion on the use of permanent hair dyes and bladder cancer risk [SCCNFP/0484/01].
- Opinion concerning the safety review of the use of certain azo-dyes in cosmetic products [SCCNFP/0495/01].
- Discussion paper on assessment strategies for hair dyes [SCCNFP/0553/02].
- Proposal for a strategy for testing hair dye cosmetic ingredients for their potential genotoxicity/mutagenicity [SCCNFP/0566/02].

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g. The inventory of cosmetic ingredients (INCI-list)

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3. SAFETY EVALUATION OF COSMETIC INGREDIENTS

3-1 INTRODUCTION

The safety of a cosmetic product in the EU is the full responsibility of the manufacturer, the first importer into the EU market or the marketer. **The safety of a cosmetic product is based on the safety of its ingredients**, the latter being evaluated by toxicological testing. The use of validated alternative methods in toxicological testing of cosmetic ingredients and finished products is compulsory for those tests for which validated alternatives exist. Deadlines for animal testing are laid down in Dir. 2003/15/EC.

The legal basis for the safety evaluation of cosmetic products, as mentioned above, can be found in Articles 2, 4.a.1 and 7a (d) of Directive 76/768/EEC and its Amendments :

Article 2 : A cosmetic product put on the market within the Community must not cause damage to human health when applied under normal or reasonably foreseeable conditions of use, taking account, in particular, of the product's presentation, its labelling, any instructions for its use and disposal as well as any other indication or information provided by the manufacturer or his authorized agent or by any other person responsible for placing the product on the Community market.

Article 4.a.1 : Without prejudice to the general obligations deriving from Article 2, Member States shall prohibit :

(a) the marketing of cosmetic products where the final formulation, in order to meet the requirements of this Directive, has been the subject of animal testing using a method other than an alternative method after such alternative method has been validated and adopted at Community level with due regard to the development of validation within the OECD;

(b) the marketing of cosmetic products containing ingredients or combinations of ingredients which, in order to meet the requirements of this Directive, have been the subject of animal testing using a method other than an alternative method after such alternative method has been validated and adopted at Community level with due regard to the development of validation within the OECD;

(c) the performance on their territory of animal testing of finished cosmetic products in order to meet the requirements of this Directive;

(d) the performance on their territory of animal testing of ingredients or combinations of ingredients in order to meet the requirements of this Directive, no later than the date on which such tests are required to be replaced by one or more validated alternative methods listed in Annex V to Council Directive 67/548/EEC … or in Annex IX to this Directive.

To be kept readily available to the Competent Authorities :

Article 7a (d) : Assessment of the safety for human health of the finished product. To that end the manufacturer shall take into consideration the general toxicological profile of the ingredients, their chemical structure and their level of exposure. …

The rationale behind Article 7a (d) is that, although there are many thousands of different cosmetic products on the market within the EU, they are all derived from fewer ingredients. Hence toxicity testing has been concentrated on ingredients, and particularly on those that are intended to react with biological matrices and therefore are of most concern for human health. This is also the basis for the lists of authorised ingredients currently covering colouring agents, preservatives and UV filters, more specifically Annexes IV, VI and VII to Dir. 76/768/EEC.

In order to fulfil the main requirements regarding consumer health protection, Article 4 of Dir. 76/768/EEC and its amendments states that :

Art. 4b : The use in cosmetic products of substances classified as carcinogenic, mutagenic or toxic for reproduction, of category 1, 2 and 3, under Annex I to Directive 67/548/EEC shall be prohibited. … A substance classified in category 3 may be used in cosmetics if the substance has been evaluated by the SCCNFP and found acceptable for use in cosmetic products.

Art. 4(1) : Without prejudice to their general obligations deriving from Article 2, Member States shall prohibit the marketing of cosmetic products containing :

a. substances listed in Annex II;
b. substances listed in the first part of Annex III, beyond the limits and outside the conditions laid down;
c. colouring agents other than those listed in Annex IV, Part I. with the exception of cosmetic products containing colouring agents intended solely to colour hair;
d. colouring agents listed in Annex IV, Part 1, used outside the conditions laid down, with the exception of cosmetic products containing colouring agents intended solely to colour hair;
e. preservatives other than those listed in Annex VI, Part 1;
f. preservatives listed in Annex VI, Part 1, beyond the limits and outside the conditions laid down, unless other concentrations are used for specific purposes apparent from the presentation of the product;
g. UV filters other than those listed in Part 1 of Annex VII;
h. UV filters listed in Part 1 of Annex VII, beyond the limits and outside the conditions laid down therein;

A series of other improvements to safeguard consumer health were introduced with the adoption of the Sixth Amendment [93/35/EEC].
These improvements oblige those responsible for placing a cosmetic product on the Community market to keep the following information readily available for the competent authorities :

a. The qualitative and quantitative composition of the product; in the case of perfume compositions and perfumes, the name and code number of the composition and the identity of the supplier;

b. The physical and chemical and microbiological specifications of the raw materials and the finished product and the purity and microbiological control criteria of the cosmetic product;

c. The method of manufacture complying with the good manufacturing practice laid down by Community law or, failing that, laid down by the law of the Member State concerned; the person responsible for manufacture or first importation into the Community must possess an appropriate level of professional qualification or experience in accordance with the legislation and practice of the Member State which is the place of manufacture or first importation;

d. Assessment of the safety for human health of the finished product. To that end the manufacturer shall take into consideration the general toxicological profile of the ingredients, their chemical structure and their level of exposure. It shall take particular account of the specific exposure characteristics of the areas on which the product will be applied or of the population for which it is intended. There shall be *inter alia* a specific assessment for cosmetic products intended for use on children under the age of three and for cosmetic products intended exclusively for use in external intimate hygiene.
Should the same product be manufactured at several places within Community territory, the manufacturer may choose a single place of manufacture where that information will be available. In this connection, and when so requested for monitoring purposes, it shall be obliged to indicate the place so chosen to the monitoring authority or authorities concerned. In this case this information shall be easily accessible;

e. The name and address of the qualified person or persons responsible for the assessment referred to in (d). That person must hold a diploma as defined in Article 1 of Council Directive 89/48/EEC in the field of pharmacy, toxicology, dermatology, medicine or a similar discipline;

f. Existing data on undesirable effects on human health resulting from use of the cosmetic product;

g. Proof of the effect claimed for the cosmetic product, where justified by the nature of the effect or product;

h. Data on any animal testing performed by the manufacturer, his agent or suppliers, relating to the development or safety evaluation of the product or its ingredients, including any animal testing performed to meet the legislative or regulatory requirements of non-member countries.

In addition, the assessment of the ingredients' toxicity has to be carried out in accordance with the principles of good laboratory practice.

Through the whole Sixth Amendment [93/35/EEC], there was a clear intention to avoid the costly duplication of toxicological studies and more importantly, the unjustifiable use of animals that would result from the routine testing of products. To that end, Article 4 of Dir. 93/35/EEC stated that assessment of the safety of use of the ingredients employed in cosmetics and of the final product, should take into account the requirements of Dir. 86/609/EEC which concerns the protection of animals used for experimental and other scientific purposes.

The "Seventh" Amendment [2003/15/EC] provides a rigid time frame regarding the application of non-animal alternative methods instead of animal testing. It imposes a prohibition of *in vivo* studies on cosmetic ingredients from 11 March 2009 on, with the exception of repeated dose toxicity, toxicokinetics and reproduction toxicity tests, which will be prohibited from 11 March 2013.

3-2 SAFETY EVALUATION PROCEDURE OF COSMETIC INGREDIENTS AS APPLIED BY THE SCCNFP

In the EU, two channels function with respect to the safety evaluation of cosmetic ingredients (Fig.1) :

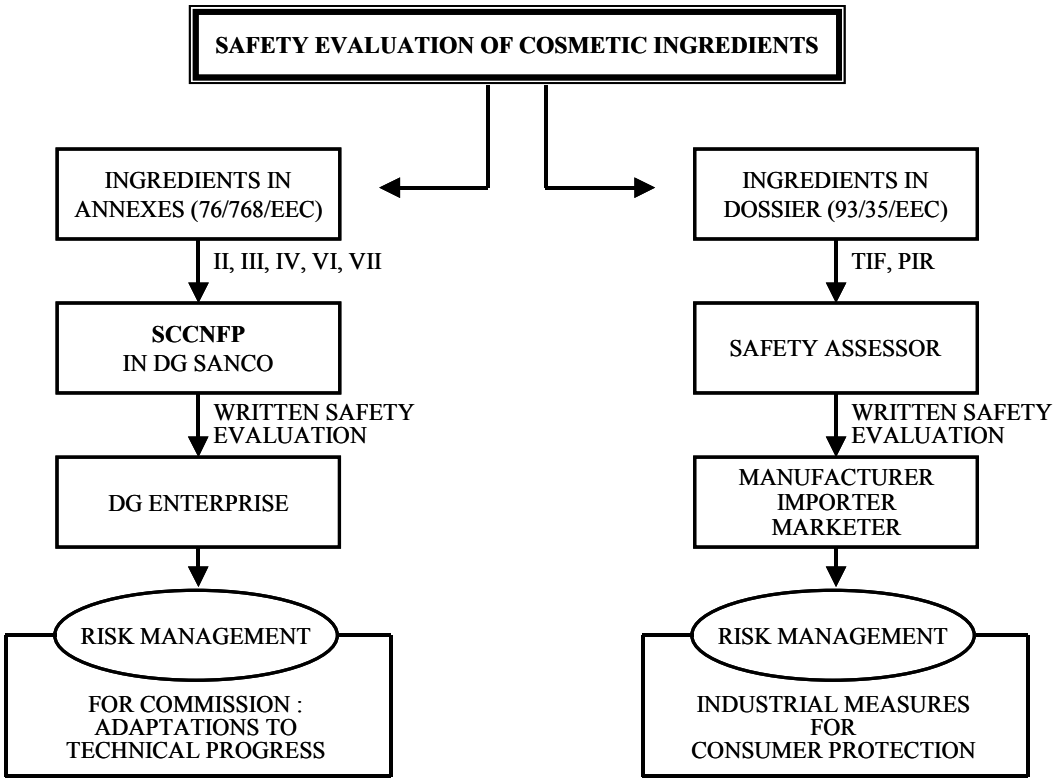

Fig.1 : Existing two ways in the safety evaluation of cosmetic ingredients in the EU.

It is primarily the substances in Annexes II, III, IV, VI and VII that fall under the responsibility of the SCCNFP. The right part of Fig.1, containing all ingredients of cosmetic products other than those of the Annexes, is the responsibility of the manufacturer through the safety assessor. In general, the **safety evaluation** of cosmetic ingredients by the SCCNFP is based upon the principles and practice of the risk assessment process [WHO 2001; European Commission 2000] usually applied for ingredients in medicinal products, pesticides, food additives, …

This risk assessment procedure is subdivided in 4 parts :

1) **Hazard identification** : based on the results of *in vivo* tests, *in vitro* tests, clinical studies, accidents, human epidemiological studies and, when available, quantitative structure activity relationship (QSAR) studies. The intrinsic physical, chemical and toxicological properties of the molecule under consideration are studied to identify whether the substance has the potential to damage human health.

2) **Dose-response assessment** : in which the relationship between the toxic response and the exposure is studied. In the case of an effect with a threshold, the dosage at which no adverse effects are observed (NOAEL), is determined. If the NOAEL is not available, the LO(A)EL is used. In the case of non-threshold carcinogens, a dose-descriptor (e.g. T_{25}) is determined.

3) **Exposure assessment** : in which the amount and the frequency of human exposure to the compound are determined (including potential specific groups at risk, e.g. children, pregnant women, etc.).

4) **Risk characterisation** : the probability that the molecule under investigation causes damage to human health and to what extent, are examined. In the case of a threshold effect, the Margin of Safety (MoS) is calculated according to the formula :

   $$MoS = \frac{NOAEL}{SED}$$ where SED represents the Systemic Exposure Dosage.

   For non-threshold effects (e.g. non-threshold carcinogenic effect) the lifetime risk is determined through the use of a dose-descriptor, defined as the calculated amount of a test substance administered daily (e.g. mg/kg body weight/day) that in the case of a non-threshold carcinogen increases the net frequency of tumours at a specific site by a certain percentage (e.g. T_{25}) [Dybing et al. 1997]. The calculation of lifetime cancer risk is described in Section 3-7.5.

Risk characterisation is followed by **risk management** and **risk communication**, which are not the tasks of the SCCNFP, but of the Commission in the case of the ingredients listed in the different Annexes (see Fig.1) [COM(97) 183].

It is beyond the scope of the "Notes of Guidance" to discuss the whole process of risk assessment. Review articles and toxicology books exist on this topic [Beck et al. 1994; Dayan 1999; Loprieno 1999; Rogiers 2002a; Masson 1999, Sanner 2001]. The aim is to highlight some key aspects in order to explain why certain data and test results should be provided in the dossiers of the ingredients presented to the SCCNFP for consideration, e.g. physical and chemical data, results of relevant toxicity studies, etc.

3-3 CHEMICAL AND PHYSICAL SPECIFICATIONS OF COSMETIC INGREDIENTS

Physical and chemical properties of ingredients are considered as crucial information, since they may be able to predict certain toxicological properties. For example, a small molecular weight (MW) hydrophobic compound is more likely to penetrate through the skin than a high MW hydrophilic compound; a highly volatile compound could cause significant inhalation exposure when present in a product applied to the skin. Physical and chemical properties also identify physical hazards of the ingredient (e.g. explosiveness, flammability). In addition, some QSAR programmes and empirical models use physical and chemical property values as inputs [Salminen 2002].
According to the SCCNFP opinion on the basic requirements for toxicological dossiers to be evaluated by the SCCNFP [SCCNFP/0633/02], the basic and minimal specifications for any ingredient should be :

1) chemical identity;
2) physical form;
3) molecular weight;
4) purity of the chemical;
5) characterisation of the impurities or accompanying contaminants;
6) solubility;
7) partition coefficient (Log P_{ow});
8) additional relevant physical and chemical specifications.

The information from points 1) to 7) must be included in each toxicological dossier. The appropriate **certificate of analysis** must be present in order to provide full characterisation of the test chemical employed to generate the data of the dossier to be considered by the SCCNFP [SCCNFP/0633/02].

In the following chapter, the methods are (where relevant) accompanied by their corresponding reference number in Annex V, Part A of Dir. 67/548/EEC [92/69/EEC].

3-3.1 Chemical identity

The precise chemical nature of the ingredient and its structural formula must be identified. The Chemical Abstracts Service (CAS) No. of the chemical, the International Nomenclature of Cosmetic Ingredients (INCI) name and the number of the European Inventory of Existing commercial Chemical Substances (EINECS), must be provided. Chemical substances introduced in the EU market after 18 September 1981 do not have an EINECS No., but have to be notified to the competent authority in the Member State in which they are manufactured or into which they are imported [Directive 67/548/EEC as amended for the 7th time by Directive 92/32/EC]. Having received this notification, the competent authority is required to carry out risk assessment of the substance for man and environment in accordance with the principles set out in Commission Directive 93/67/EC. New substances are recorded in the European LIst of Notified Chemical Substances (ELINCS database) and the ELINCS No. of the new substances must be identified in the dossier to be submitted for safety evaluation.

With regard to ingredients that cannot be identified in terms of their structural formula, sufficient information should be provided on the method of preparation (including all physical, chemical, enzymatic, biotechnological and microbiological steps) and the material used in their preparation to assess the probable structure and activity of the compound.

For the safety evaluation of a natural ingredient (extract), complete information should be provided on the origin of the raw material (e.g. part of plant), extraction method and any additional purification steps used (see also section 3-6.2).

In the case of a preparation used as "raw material", all substances must be given in the qualitative and the quantitative formula. These could be : main components, preservatives, antioxidants, chelators, buffering agents, solvents, other additives and additional external contamination.

When a salt or ester of a substance will be used as cosmetic ingredient, this must be clearly specified in the dossier. The physical and chemical properties of the specific salts/esters must be provided. And the same specific substances must be used in the toxicological studies performed for the safety evaluation. Deviations should be justified.

3-3.2 Physical form

A description of the physical form should be given : powder, paste, gel, liquid, …

3-3.3 Molecular weight

The MW of each substance should be given in Daltons. In the case of preparations, the MW must be given for each of the constituents.

3-3.4 Purity of the chemical

The degree of purity must be clearly defined. The validity of the analytical methodology used, must be shown.
The substances used in physical and chemical tests, toxicity studies, etc., mentioned in the dossier, must be representative of the substances present in commercial products.

3-3.5 Characterisation of the impurities or accompanying contaminants

In addition to the purity of the substance under consideration, an identification of the nature of significant impurities that may be present must be stated, along with their concentration.

Small changes in the nature of impurities can considerably alter the toxicity of substances. In general, **results of safety studies on a particular substance are only relevant when they refer to that substance used, with its own specific purity and impurity patterns**. The scientific validity of tests performed on batches of the substance with diverging purities is questionable. Therefore, the manufacturer must ensure that there are no other impurities nor an increase in chemically defined or technically unavoidable (potentially affecting the safety of the finished products) present in the representative commercial material and samples used for its physical and chemical studies and hazard identification.

3-3.6 Solubility

The solubility [EC A.6] of the ingredient in water and/or in any other relevant organic solvent should be stated (in g/l at ...°C). Some substances are sparingly soluble or insoluble in aqueous medium.

3-3.7 Partition coefficient (Log P_{ow})

The n-octanol / water partition coefficient [EC A.8] should be given (at …°C).
In case of a calculated value, the method should be specified.

3-3.8 Additional relevant physical and chemical specifications

A typical physical and chemical data set consists of :

- physical state (solid, liquid, gas)
- organoleptic properties (colour, odour, taste if relevant)
- solubility properties [EC A.6] in water and relevant organic solvents (at ..°C)
- partition coefficient [EC A.8] (Log P_{ow}, at ..°C), if applicable
- flash point [EC A.9]
- physical properties depending on the physical state :
  - for liquids : boiling point [EC A.2], density [EC A.3] (at ..°C), pK_a (at ..°C), viscosity (at ..°C), vapour pressure [EC A.4] (at ..°C), ...
  - for solids : general appearance (crystal form, amorphous, ...), melting point [EC A.1], pK_a (..% in ..., at ..°C), ...
  - for gases : density [EC A.3] (at ..°C), ignition point [EC A.15], …
- in case of a UV light absorbing ingredient, the UV light absorption spectrum of the compound should be included

3-4 RELEVANT TOXICITY STUDIES ON COSMETIC INGREDIENTS

The determination of the toxic potential of a cosmetic ingredient is based on a series of toxicity studies and forms part of the hazard identification. The latter is the first step in its overall safety evaluation.
At present, the majority of these toxicological tests involve the use of animals, as is also the case for other chemical substances. Traditionally, toxicological data relevant for man have been obtained by investigating the toxicological profiles of the substances under consideration on animals, using the same exposure route as in man (topical, oral or inhalation route).
Single dose animal studies, usually carried out with high concentrations of the test compound, allow determination of LD_{50}-values, which form the basis for the classification of e.g. dangerous substances [2001/59/EC]. Repeated dose toxicity studies, usually performed with lower concentrations and involving daily administration/exposure for a long period of time (e.g. 28 days / 90 days / 24 months), allow for the determination of the so-called no-observed adverse effect level (NOAEL), which is used in the calculation of the Margin of Safety (MoS). These studies also give an indication on target organs, mechanisms of action, etc.
Carcinogenicity studies are usually performed with mice and rats for a period of 18 months to 24 months.

One of the scientific objectives of the EU is the development and validation of alternative methods that can provide an equivalent level of information as current animal tests, but which use fewer animals, cause less suffering or avoid the use of animals completely (3R-strategy of refinement, reduction and replacement).
In this respect, significant refinement and reduction improvements have been made to existing *in vivo* guidelines and a number of replacement guidelines have been developed. The latter are based on *in vitro* methods, more specifically in the field of skin corrosion, photomutagenicity, phototoxicity, and dermal absorption. However, due to a variety of reasons, including the complexity of the vertebrate organism, there are presently no validated *in vitro* alternative methods for the repeated dose animal toxicity studies available, nor are there relevant proposals ready for prevalidation/validation [Worth et al. 2002; Rogiers 2002b].

The SCCNFP stresses the fact that it is aware that valuable toxicity data are available for ingredients that have been subject to the chemical substances notification procedure [92/32/EEC]. Although this Directive recognises Art. 7.2* of Dir. 86/609/EEC on the protection of laboratory animals, until now the Competent Authorities have not readily accepted alternative test methods that have not been taken up in Annex V, Part B of the Dangerous Substances Directive and its relevant adaptations to technical progress [67/548/EEC, 84/449/EEC, 88/302/EEC, 92/69/EEC, 96/54/EC, 2000/32/EC, 2000/33/EC and 2001/59/EC].

For cosmetic ingredients, the SCCNFP accepts, besides validated alternative methods, also "valid" methods. These have not necessarily gone through the complete validation process, but the Committee considers these methods acceptable when they have a sufficient amount of scientific data proving their relevance and reliability. According to the Sixth Amendment [93/35/EEC] to the Cosmetic Products Directive, the evaluation of the safety for human health also has to be carried out in accordance with the principles of Good Laboratory Practice laid down in Council Directive 87/18/EEC. All possible deviations from this set of rules must be explained and scientifically justified [SCCNFP/0633/02].

This chapter describes the currently used animal tests and/or their existing alternatives. Every method is referred by its reference number in Annex V, Part B of Dir. 67/548/EEC and by its OECD (Organisation for Economic Co-operation and Development) number.

3-4.1 Acute toxicity

The term "acute toxicity" is used to describe the adverse effects on health, which may result from a single exposure to a substance via the oral, dermal or inhalation route [ECB 2003].

The *in vivo* acute oral toxicity test was originally developed to determine the LD_{50}-value of the compound under investigation. In the dangerous substances legislation, this LD_{50}-value triggers the classification of the compound [2001/59/EC].

* **Art.7.2** : "An experiment shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonably and practically available."

The original test method [EC B.1, OECD 401] involving between three and five dosage groups each comprising 5 to 10 animals, has been deleted [2001/59/EC] and replaced by the following alternative methods :

1) The **fixed dose method** [EC B.1 bis, OECD 420] abandons lethality as and endpoint and is designed not to cause death, marked pain or distress to the animals and thereby is a useful refinement alternative method to EC B.1 / OECD 401.
2) The **acute toxic class method** [EC B.1 tris, OECD 423] does not aim to calculate a precise LD_{50}-value, but allows the determination of a range of exposure dosages where lethality is expected. The test follows a complex stepwise dosage scheme and may consequently take longer than the original EC B.1 / OECD 401 and the alternative EC B.1 bis / OECD 420 method. Nevertheless it offers, as a main and important advantage, a significant reduction in the number of animals tested.
3) The **up-and-down procedure** [OECD 425] allows an estimation of the LD_{50}-value and confidence intervals, and the observation of signs of toxicity. The guideline significantly reduces the number of animals used in comparison to Guideline EC B.1 / OECD 401.

Usually acute toxicity data of cosmetic ingredients are already available as a result of compliance with the provisions of the seventh amendment to Directive 67/548/EEC on the notification, classification and labelling of dangerous substances [92/32/EEC].

3-4.2 Irritation and corrosivity

1) Skin irritation and skin corrosivity

Skin irritation tests have been developed to assess the potential of a certain substance to cause redness and/or oedema after a single topical application. There are to date no validated alternative methods capable of replacing the classical Draize *in vivo* skin irritation test [EC B.4, OECD 404].
Several *in vitro* skin irritation tests are under validation. It is hoped that an acceptable test will become available soon.

Skin corrosion tests assess the potential of a substance to cause irreversible damage to the skin, namely visible necrosis through the epidermis and into the dermis, following the application of a test substance for the duration period of 3 minutes up to 4 hours. Corrosive reactions are typified by ulcers, bleeding, scabs, and, by the end of observation at 14 days, by discolouration due to blanching of the skin, complete areas of alopecia, and scars [OECD 404]. Corrosivity is not a feature one expects to occur with cosmetics, but occasionally could occur after a manufacturing mistake or misuse by the consumer. On the other hand, a cosmetic ingredient that has the intrinsic property to be corrosive, is not necessarily excluded for use in cosmetics. It very much depends on its final concentration in the cosmetic product, the presence of "neutralising" substances, the excipient used, the exposure route, the conditions of use, etc.

For skin corrosion testing, actually 3 validated alternatives are taken up in Annex V to Dir. 67/548/EEC :

1) The "*In vitro* Skin Corrosion : Rat Skin Transcutaneous Electrical Resistance test" uses excised rat skin as a test system and its electrical resistance as an endpoint [EC B.40, Draft OECD 430].
2) EPISKIN™ and 3) EpiDerm™ are two commercialised human skin model tests consisting of reconstructed human epidermal equivalent using cell viability (MTT-test) as an endpoint [EC B.40, Draft OECD 431].

The Corrositex™ test, which uses penetration of test substances through a hydrogenated collagen matrix (biobarrier) and supporting filter membrane, represents another corrosivity test. Although it passed the ECVAM (European Centre for the Validation of Alternative Methods) Scientific Advisory Committee (ESAC), it has not been taken up in the EU legislation. It is considered to be only useful for acids and bases [ESAC 2000].

For skin irritation testing, some general refinement provisions have been included in the test protocol [EC B.4, OECD 404], such as :

1) A substance with a pH below 2.0 or above 11.5, should not be tested, due to its suspected corrosivity.
2) A substance found to be corrosive in one of the alternative corrosivity tests (taken up in Annex V of Dir. 67/548/EEC), should not be tested in the Draize test.

2) Mucous membrane irritation

Eye irritation tests have been developed to assess the potential of a certain substance to cause chemosis, discharge and/or redness to the conjunctiva, swelling of the iris and/or opacity to the cornea, after a single application. There are presently no validated alternative methods replacing the classical Draize *in vivo* eye irritation test [EC B.5, OECD 405]. ECVAM is currently involved in the validation of alternative eye irritation methods. It is generally believed that a battery of alternative tests will be required for the assessment of eye irritation since multiple mechanisms of eye irritation exist.

Nevertheless, some general reduction provisions have been included in the currently existing test protocol [EC B.5, OECD 405], such as :

1) Substances with a pH below 2.0 or above 11.5 should not be tested.
2) Substances suspected or proven to be irritating to the skin, should not be applied into the eye.

As a "valid" alternative method (not formally validated), the BCOP-test (Bovine Cornea Opacity-Permeability test) appears acceptable for neutral organic chemicals [SCCNFP/0546/02]. It provides quantitative data on the opacity and permeability of the cornea of slaughterhouse animals (bovine, chicken, rabbit) after treatment with the compound or product under investigation. It is more sophisticated than the enucleated eye test (bovine eyes) that only provides a subjective score.
The RBC (Red Blood Cell) and NRU (Neutral Red Uptake) tests are useful for testing of surfactants. For alcohols and esters, no good methodologies are yet available [SCCNFP/0546/02].

Finally, the HET-CAM test (Hen's Egg Test - ChorioAllantoic Membrane) [Gilleron et al. 1996] is a "valid" alternative method often used in screening studies for finished cosmetic products. It has not been formally validated, but is taken up in the legislation of some EU Member States (e.g. France).

3-4.3 Skin sensitisation

A skin sensitiser is an agent that is able to cause an allergic response in susceptible individuals. The consequence of this is that following subsequent exposure via the skin, the characteristic adverse health effects of allergic contact dermatitis may be provoked [ECB 2003]. As yet, there is not a validated *in vitro* test method accepted for skin sensitisation.

There are three common *in vivo* laboratory animal test methods to evaluate the potential of a substance to cause skin sensitisation :

1) The Local Lymph Node Assay (LLNA) [OECD Draft Guideline 429] uses an inbred strain of mice, and is based on the extent of stimulation of proliferation of lymphocytes in regional lymph nodes draining the site of application of the test substance. It is an objective method giving the result as a stimulation index (SI), which is the ratio of stimulation caused by the test substance in animals versus that in vehicle treated control animals. The test substance is applied openly to the dorsum of the ear in a suitable vehicle, and the use of Freund's complete adjuvant as an immune enhancer causing local skin inflammation is avoided. The LLNA is an alternative method on mice that refines the methodology in comparison with the traditional guinea pig-based models as described below. It has, however, not been taken up in Annex V to Dir. 67/548/EEC.
2) The Magnusson Kligman Guinea Pig Maximisation Test (GPMT) [EC B.6, OECD 406] is an adjuvant-type test, which means that the allergic response is potentiated by intradermal injection of the test substance with and without Freunds Complete Adjuvant. The GPMT is considered equal in sensitivity compared to the LLNA. The test result is based on the challenge response to a non-irritant patch test with the test substance. Thus, the test mimics the "real-life" development of allergic contact dermatitis. The method allows repeated challenges, cross reactivity and vehicle effect studies.
3) The Buehler test [EC B.6, OECD 406] is a non-adjuvant technique that involves topical application only. The method is less sensitive compared to the GPMT. Scientific justification should be given in case the Buehler test is used.

3-4.4 Dermal / percutaneous absorption

Human exposure to cosmetic ingredients occurs mainly via the skin. In order to reach the circulation (blood and lymph vessels) cosmetic ingredients must cross a number of cell layers of the skin, whereby the rate-determining layer is considered to be the stratum corneum (SC). A number of factors play a key role in this process, including the lipophilicity of the compounds, the thickness and composition of the SC (body site), the duration of exposure, the amount of topically applied product, the concentration of the compounds considered, occlusion, etc. [for review see Schaefer et al. 1996; ECETOC 1993; Howes et al. 1996].

Dermal / percutaneous absorption studies can be performed *in vivo* or *in vitro*. The *in vivo* dermal / percutaneous absorption method is among others described in Draft OECD Guideline 427 combined with the "Draft Guidance Document for the conduct of skin absorption studies" [OECD 2000]. The *in vitro* study has been summarized in OECD Guideline 428 and again, more detail is given in the above-mentioned OECD Guidance Document, which provides useful additional guidance for both types of studies. According to the 7th Amendment to Dir. 76/768/EEC [2003/15/EC], the *in vivo* study will be prohibited for cosmetic ingredients from 11 March 2009 on.

The *in vivo* and *in vitro* dermal / percutaneous absorption studies have been described by several international bodies [ECETOC 1993, US EPA 1996a, OECD 2000], using such a wide variety of terms, that confusion is possible. It therefore seems appropriate to define two important terms used in the actual SCCNFP guidance document, primarily considering *in vitro* methods :

- *SC or dermal adsorption* : (= substantivity) amount of topically applied test substance present in or sticking to the SC. It is considered not to be systemically available and is excluded from risk assessment [based on Diembeck et al. 1999]
- *Dermal / percutaneous absorption* : amount of dermally applied substance remaining in the residual skin (excluding the stratum corneum) plus the amount of dermally applied substance which has transpassed the skin and is detected in the receptor fluid. The sum is considered to be systemically available (= dermal bioavailability) [based on OECD 2000, Diembeck et al. 1999, ECETOC 1993].

The amount of topically applied substance found to have transpassed the SC and entered into deeper skin layers, will be considered by the SCCNFP as relevant for safety evaluation (calculation of the Margin of Safety), as long as the application was performed mimicking in-use conditions. The amount is expected to enter the circulatory system unless irreversible binding in the epidermis and/or dermis has been demonstrated.

A year before the publication of Draft OECD Guideline 428, the SCCNFP adopted a set of basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients [SCCNFP/0167/99]. OECD 428 addresses percutaneous absorption from a broad point of view by mentioning *in vivo* methods besides *in vitro* testing and providing specifications for agricultural products, industrial chemicals and cosmetics. In contrast, the above mentioned SCCNFP basic criteria are exclusively focused on the *in vitro* testing of cosmetic ingredients. They have been updated in October 2003 [SCCNFP/0750/03] and should be consulted whenever cosmetic dermal / percutaneous absorption studies are being performed / planned.

The test formulations used should be an adequate representation of the final cosmetic product(s). If the test substance is used in different types of cosmetic products, it is necessary to perform *in vitro* testing on more than one type of formulation. Moreover, concentrations of ingredients tested must be chosen thus that they are close to (but not less than) the concentrations claimed for acceptance [SCCNFP/0167/99].

In case adequate dermal absorption studies are not available, a default value for dermal absorption of 100% is applied in the calculation of the Margin of Safety (MoS).

3-4.5 Repeated dose toxicity

Repeated dose toxicity comprises the adverse general (excluding reproductive, genotoxic and carcinogenic effects) toxicological effects occurring as a result of repeated daily dosing with, or exposure to, a substance for a specific part of the expected lifespan of the test species [ECB 2003].

The following *in vivo* repeated dose toxicity tests are available :

1) - Repeated dose (28 days) toxicity (oral) [EC B.7, OECD 407]
   - Repeated dose (28 days) toxicity (dermal) [EC B.9, OECD 410]
   - Repeated dose (28 days) toxicity (inhalation) [EC B.8, OECD 412]

2) - Sub-chronic oral toxicity test : Repeated dose 90-day oral toxicity study in rodents [EC B.26, OECD 408]
   - Sub-chronic oral toxicity test : Repeated dose 90-day oral toxicity study in non-rodents [EC B.27, OECD 409]
   - Sub-chronic dermal toxicity study : 90-day repeated dermal dose study using rodent species [EC B.28, OECD 411]
   - Sub-chronic inhalation toxicity study : 90-day repeated inhalation dose study using rodent species [EC B.29, OECD 413]

3) - Chronic toxicity test [EC B.30, OECD 452]

The 28-day and 90-day oral toxicity tests in rodents are the most commonly used repeated dose toxicity tests and often give a clear indication on target organs and type of systemic toxicity.
The inhalation route is only rarely used in repeated dose toxicity testing due to the complex study design accompanying this kind of toxicity trials, as well as to the lack of relevance of this route of repeated exposure for the majority of cosmetic products.

The objective of chronic toxicity studies is to determine the effects of a test substance in a mammalian species following repeated exposure during a period covering the whole lifespan of the animals. In these tests, effects which require a long latency period or which are cumulative, become manifest.

As already mentioned, for repeated-dose toxicity testing, currently no validated or generally accepted alternative methods are available for replacing animal testing. There have been some serious efforts in the domains of e.g. neurotoxicity and nephrotoxicity, but to date, no method or screening battery has been formally (pre-)validated [SCCNFP/0546/02].

In the notification process of dangerous substances, repeated dose toxicity studies are required when the substance under consideration is produced or imported in amounts exceeding 1 tonne/year [92/32/EEC].
In the case of the development of cosmetic ingredients which have specific biological properties and which will come into contact with human skin for a long period of time, the SCCNFP is convinced that evaluation of the systemic risk is a key element in evaluating the safety of these new ingredients, irrespective of the tonnage-linked and possibly restricted requirements imposed by the Dangerous Substances Directive [67/548/EEC].

Therefore the SCCNFP considers that in certain cases the use of animal long-term experiments to study one or more potential toxic effects remains a scientific necessity. It is self-evident that animal use should be limited to a minimum, but never at the expense of consumer safety. The "7th Amendment" [2003/15/EC] to the Cosmetic Directive 76/768/EEC allows up to 11 March 2013 for the development of validated alternative tests for repeated exposure.

3-4.6 Mutagenicity/genotoxicity

In the safety evaluation of cosmetic ingredients it is necessary to address the potential effect of "mutagenicity".

Mutagenicity refers to the induction of permanent transmissible changes in the amount or structure of the genetic material of cells or organisms. These changes may involve a single gene or a gene segment, a block of genes or whole chromosomes. Effects on whole chromosomes may be structural and/or numerical [ECB 2003].

It has been demonstrated that, moreover, mutations can arise from damages induced in the DNA molecules that are not considered to be a consequence of "genotoxicity" effects.

Genotoxicity is a broader term and refers to potentially harmful effects on genetic material that are not necessarily associated with mutagenicity. Thus, tests for genotoxicity include those providing an indication of induced damage to DNA (but not direct evidence of mutation) via effects such as unscheduled DNA synthesis (UDS), sister chromatid exchange (SCE), DNA strandbreaks, DNA adduct formation or mitotic recombination (MR), as well as tests for mutagenicity [ECB 2003].

Several *in vitro* and *in vivo* tests are available and have been described in OECD Guidelines as well as in Annex V to Directive 67/548/EEC. As a general recommendation, when testing for the genotoxic/mutagenic potential of a cosmetic ingredient, the SCCNFP is of the opinion that a combination of two *in vitro* tests is necessary, namely :

1) a bacterial reverse mutation test (the Ames test) [EC B.13/14, OECD 471]
   OR
   an *in vitro* mammalian cell gene mutation test* [EC B.17, OECD 476]
   (only for specific chemicals, always accompanied by a scientific justification),

   WITH

2) an *in vitro* mammalian cell chromosome aberration test [EC B.10, OECD 473],

These tests usually are considered to provide sufficient evidence of mutagenic and/or genotoxic potential. It is, however, possible that the actual scheme may change as a consequence of new insights into the applicability of the *in vitro* micronucleus test on mammalian cells [BfR 2002, Gocke et al. 2000, Emea 2002].

It is essential that all tests are conducted according to rigorous protocols in order to maximise the potential for detecting a mutagenic response, to ensure that negative results can be accepted with confidence and that results are comparable when tests are conducted in different laboratories.

* Currently the preferred choice is the mouse lymphoma assay; the CHO HPRT (Hypoxanthine-guanine PhosphoRibosyl Transferase) assay has more shortcomings.

For most cosmetic ingredients, *in vivo* tests are not considered imperative. It is only in very rare cases that *in vivo* tests are able to pick up mutagenic potentials that were not observed at the *in vitro* level. Animal experiments are only deemed to be essential for human and animal food and drugs material. The strongest argument for the need for animal experiments is that activation could possibly take place *in vivo* which may not be simulated by the addition of S9-mix. Therefore, more variation in the metabolizing system at the *in vitro* level would be preferable [BfR 2002].

There are several reasons why mutagenicity testing beyond the base level may be required. Normally, if there is clear structural alert for mutagenicity or when some concern is raised by positive results from *in vitro* tests, further testing may be justified, e.g. micronucleus test in mammalian cells.

Before undertaking any *in vivo* testing, a thorough review is needed of the *in vitro* test results of the substance (with its toxicokinetic profile), available information on its chemistry, impurities and toxicological profile, as well as data on analogous ingredients.
It is obvious that a particular *in vivo* test should be conducted only when it can be reasonably expected from all the properties of the test substance and the proposed test protocol that the specific target tissue will be adequately exposed to the test substances and/or its metabolites.

In June 2003, a new strategy for testing oxidative hair dye ingredients for their potential genotoxicity/mutagenicity/carcinogenicity was adopted by the SCCNFP [SCCNFP/0720/03]. This strategy imposes six *in vitro* tests instead of the two mentioned above, viewing the fact that several permanent hair dyes contain aromatic amines or may form them during the oxidative reaction. Also human exposure is expected to be extensive in certain cases (e.g. home usage).

3-4.7 Carcinogenicity

Substances are defined as carcinogenic if they induce tumours (benign or malignant) or increase their incidence, malignancy or shorten the time of tumour occurrence when they are inhaled, ingested, dermally applied or injected [ECB 2003].

The most commonly performed carcinogenicity tests are :

1) Carcinogenicity test [EC B.30, OECD 452]
2) Combined chronic toxicity / carcinogenicity test [EC B.33, OECD 453]

Genotoxic carcinogens are chemicals for which the most plausible mode of carcinogenic action includes the consequences of genotoxic effects [ECB 2003]. When there is structural alert for carcinogenicity or positive results in *in vitro* mutagenicity tests, an *In vitro* Syrian Hamster Embryo (SHE) Transformation Test [OECD 1996] may be needed, irrespective of the fact whether it concerns genotoxic or non-genotoxic substances.

As far as genotoxic compounds are concerned, *in vitro* mutagenicity tests are quite well developed. Tests for detecting non-genotoxic carcinogens constitute another issue. Therefore, *in vivo* rodent studies will remain necessary in specific cases.

3-4.8 Reproductive toxicity

The term "reproductive toxicity" is used to describe the adverse effects induced (by a substance) on any aspect of mammalian reproduction. It covers all phases of the reproductive cycle, including impairment of male or female reproductive function or capacity and the induction of non-heritable adverse effects in the progeny such as death, growth retardation, structural and functional effects [ECB 2003].

The most commonly performed *in vivo* reproduction toxicity studies are :

1) Two-generation reproduction toxicity test [EC B.35, OECD 416]
2) Teratogenicity test - rodent and non-rodent [EC B.31, OECD 414]

At the OECD level, there also exists a combined "Reproduction/Developmental Toxicity Screening Test" [OECD 421], which has to date not been taken up in Annex V to Dir. 67/548/EEC.

Since the field of reproductive toxicity is very complex, it is expected that the various stages cannot be mimicked using one alternative method. Three alternative methods, restricted to the embryotoxicity area, have been developed :

1) The Whole Embryo Culture test (WEC)
2) The MicroMass test (MM)
3) The Embryotoxic Stem cell Test (EST)

The last two tests were considered scientifically valid by ESAC for placing the substance under consideration into one of the 3 following categories : non-embryotoxic, weak/moderate-embryotoxic or strong-embryotoxic. The WEC test is considered scientifically valid only for identifying strong embryotoxic substances [ESAC 2001].
These 3 alternative embryotoxicity tests, which are further refined with ECVAM support, have recently been discussed within the SCCNFP and are considered to be useful in the CMR strategy for screening out embryotoxic substances. However, more data on positive reacting compounds remain necessary.

3-4.9 Toxicokinetic studies

The term "toxicokinetic studies" is used to describe the time-dependent fate of a substance within the body. This includes absorption, distribution, biotransformation and/or excretion. The term "toxicodynamics" means the process of interaction of chemical substances with target sites and the subsequent reactions leading to adverse effects [ECB 2003].

The protocols for toxicokinetics [EC B.36, OECD 417] are designed to elucidate particular aspects of the toxicity of the substance under test. The results may assist in the design of further toxicity studies and their interpretation. Moreover, after dermal absorption of a substance under consideration, its metabolic fate can have an important effect on its toxic potential, its distribution in the body and its excretion. Therefore, in specific cases, *in vivo* or *in vitro* biotransformation studies are required to prove or to exclude certain adverse effects. Several *in vitro* models (e.g. hepatocytes in suspension or culture) are suitable for biotransformation studies, however, none of these models has been validated [Blaauboer et al. 1994, Coecke et al. 1999].

Information on chemical structure (e.g. QSAR) and physical and chemical properties (e.g. $logP_{ow}$) may also provide an indication of the absorption characteristics by the intended route of administration, metabolism and tissue distribution. There may also be information on toxicokinetic parameters from preceding toxicity studies.

Finally, toxicokinetic studies are of importance in extrapolating both *in vitro* and *in vivo* animal data to man.

3-4.10 Photo-induced toxicity

1) Phototoxicity (photoirritation) and photosensitisation

The "3T3 Neutral Red Uptake Phototoxicity Test (3T3 NRU PT)" is an *in vitro* method based on a comparison of the cytotoxicity of a chemical when tested in the presence and in the absence of exposure to a non-cytotoxic dose of UVA/visible light.
In 1998, the SCCNFP recommended the use of this *in vitro* method for the determination of the photoxicological/photoirritative profile of all UV light absorbing chemicals and especially for those cosmetic ingredients to be used as UV filters [SCCNFP/0069/98].
In 2000, the 3T3 NRU PT test was formally validated and subsequently taken up in Annex V to Dir. 67/548/EEC [EC B.41, Draft OECD 432], making its use mandatory for testing for phototoxic potential.

The reliability and relevance of the *In vitro* 3T3 NRU Phototoxicity Test was recently evaluated for a number of substances with a chemically different structure [Spielmann et al. 1998], including UV filters used as cosmetic ingredients. The test was shown to be predictive of acute phototoxicity effects in animals and humans *in vivo*. However, it is not designed to predict other adverse effects that may arise from combined actions of a chemical and light, e.g. it does not address photoclastogenicity/ photomutagenicity, photoallergy or photocarcinogenicity.

Presently, no *in vitro* methods for detection of photosensitisation are available. Nevertheless, it is expected that chemicals showing photoallergic properties, are likely to give positive reactions in the 3T3 NRU PT test [2000/33/EC].

2) Photomutagenicity / Photoclastogenicity

In 1990 the SCC adopted guidelines for testing the photomutagenicity / photogenotoxicity of UV radiation absorbing cosmetic ingredients.

The SCCNFP has recommended that the test protocols used by COLIPA (European Cosmetic Toiletry and Perfumery Association) be the subject of a validation study. This recommendation has not yet been taken up because of the difficulty of planning a validation study in the absence of *in vivo* reference data. In the case of photomutagenicity/photogenotoxicity, in view of the established biological mechanisms (alteration of genes, chromosomes, DNA sequences), *in vivo* reference data may not be necessary.

In 1999, the OECD was already discussing Guidelines for photomutagenicity, but at present no results are available.

In the meantime, the SCCNFP is evaluating the individual photomutagenicity/genotoxicity tests and their scientific value on a case-by-case basis, keeping in mind the general provisions for the classical battery of mutagenicity/genotoxicity tests mentioned in 3-4.6.

3-4.11 Human data

Cosmetic products are developed to be applied to human skin and external mucosa and to be used by the general public. Occasionally, undesirable side effects, both local and systemic, may occur. Local reactions may be, among others, irritation, allergic contact dermatitis, contact urticaria and sunlight-, especially UV light-, induced reactions. Skin and mucous membrane irritation are the most frequently observed reactions.
Although it is inconceivable that tests in human volunteers would replace animal tests, it is known that tests in animals and alternative methods are of limited predictive value with respect to human exposure. Therefore, a skin compatibility test with human volunteers, confirming that there are no harmful effects when applying a cosmetic product for the first time to human skin or mucous membranes, may be needed scientifically and ethically.
It is self-evident that such a test can only be envisaged provided that the toxicological profiles of the ingredients, based on animal testing and/or the use of alternative methods, are available and pose no problem. A high degree of safety is to be expected. Finished cosmetic products are usually tested in small populations to confirm their skin and mucous membrane compatibility, as well as their cosmetic acceptability (= fulfilment of in-use expectations).
The general ethical and practical aspects related to human volunteer compatibility studies on finished cosmetic products, are described in Doc. SCCNFP/0068/98, Final and SCCNFP/0245/99, Final.

A separate SCCNFP opinion addresses the conduct of human volunteer testing of potentially cutaneous irritant (mixtures of) cosmetic ingredients [SCCNFP/0003/98]. Ethical and practical considerations are discussed with a specific focus on irritancy.

Finally, an SCCNFP opinion has been issued concerning the predictive testing of potentially cutaneous sensitising cosmetic (mixtures of) ingredients [SCCNFP/0120/99].
These types of tests are much more controversial than the irritancy tests, since predictive human sensitisation tests involve attempts to induce a long lasting or permanent immunologic sensitisation in the individual. Therefore, serious ethical questions arise. In spite of many years of experience with human sensitisation tests, very limited scientific information is available in the literature regarding the consequences involved for the human volunteers who have developed a patch test sensitisation during such a test. Due to the uncertainties mentioned above, it is the opinion of the SCCNFP that predictive human sensitisation tests should not be carried out without a better understanding of the immunologic background and mechanisms underlying positive reactions in these studies with human beings.

At present, no validated replacement method for predicting skin sensitisation exists. Only a "valid" refinement test, the LLNA, is available.

3-5 TOXICOLOGICAL REQUIREMENTS FOR INCLUSION OF A SUBSTANCE IN ONE OF THE ANNEXES TO DIR. 76/768/EEC (WHICH ARE EVALUATED BY THE SCCNFP)

3-5.1 General toxicological requirements

When a cosmetic ingredient dossier is submitted for evaluation by the SCCNFP, the manufacturer should provide the Commission with the information set out below :

1. *Acute toxicity (if available);*
2. *Irritation and corrosivity;*
3. *Skin sensitisation;*
4. *Dermal / percutaneous absorption;*
5. *Repeated dose toxicity;*
6. *Mutagenicity / genotoxicity;*
7. *Carcinogenicity;*
8. *Reproductive toxicity;*
9. *Toxicokinetics;*
10. *Photo-induced toxicity;*
11. *Human data.*

In general, points 1. to 6. are considered the minimal base set requirements. However, when considerable oral intake is expected or when the data on dermal / percutaneous absorption indicate a considerable penetration of the ingredients through the skin (taking into account the toxicological profile of the substance and its chemical structure), points 7., 8. and 9. may become necessary, as well as specific additional genotoxicity and/or mutagenicity data. Photo-induced toxicity data (10.) are specifically required when the cosmetic product is expected or intended to being used on sunlight-exposed skin.
Human data (11.) are extremely useful and should be included whenever available. Nevertheless, the use of human volunteers in the confirmatory testing of potentially **cutaneous irritant** cosmetic ingredients or mixtures of ingredients are subjected to ethical concerns. The use of human volunteers in the predictive testing of potentially **cutaneous sensitising** cosmetic ingredients or mixtures of ingredients, as a contribution to human safety is questionable in comparison with animal testing. Moreover, in these studies a risk for human volunteers cannot be excluded and there is still a lack of information on the severity and frequency of adverse effects [SCCNFP/0633/02].
There may be cases for which it is neither necessary nor technically possible to provide the information mentioned above : in such cases **a scientific justification** must be given.

Safety data can be obtained by means of studies conducted in accordance with guidelines reported in Annex V, Part B of the Dangerous Substances Directive and its relevant adaptations to technical progress, and complying with the principle of Good Laboratory Practice (Directive 87/18/EEC); or by means of adequate and acceptable scientific methods. All possible deviations from this set of rules must be **explained and scientifically justified**.

When study results are submitted, a declaration should be made that the tests involved were conducted using a substance with a comparable purity/impurity profile and the same physical and chemical characteristics of that to be included in the finished cosmetic product [SCCNFP/0633/02]**.**

Stability of the test substance under experimental conditions is of prime importance for the interpretation of test results. The stability of the test material should therefore be reported.

According to Art.7 of Council Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes, an animal study shall not be performed if another scientifically satisfactory method of obtaining the result sought, not entailing the use of an animal, is reasonable and practically available.
Validated *in vitro* methods for the assessment of potentially cutaneous irritants, sensitising cosmetic ingredients or mixtures of ingredients are currently not available. However, "valid" methods exist, which are particularly useful for testing of the finished cosmetic product. Animal studies to predict the above said effects are reliable and well documented in the scientific literature.

Finally, the SCCNFP highlights the important requirement of ensuring that files for evaluation are **complete** when submitted. The applicant must ensure this **by signature**.

Together with the relevant experimental investigations, the following information should also be available :

- any report on epidemiological and/or observational experiences;
- description of all available ecological and environmental effects of the respective substance/compound/preparation;
- all relevant published literature;
- a description of the bibliographical methods used;
- any useful finding to the applicant's best ability;
- any "grey material" available elsewhere.

Subsequently, any new information acquired by industry and/or relevant agencies, should be transmitted to the Commission for review [SCCNFP/0461/01].

3-5.2 Annex II

Annex II to Dir. 76/768/EEC is a list containing substances that must not form part of the composition of cosmetic products.

3-5.3 Annex III

Annex III is defined as a list of substances that are allowed to be used in cosmetic products, but only subject to the restrictions and conditions laid down.
This Annex contains substances that have been identified as posing a possible risk to human health when used in cosmetic products above the defined maximum authorized concentration in the finished product or where certain applications need to be restricted.
For inclusion in Annex III, the general requirements as defined in 3-5.1, apply.

3-5.4 Annex IV

Annex IV constitutes a list of colouring agents permitted for use in cosmetic products.
A number of these colourants have a wide use in food and have been declared as safe for use for many years, while on others clear restrictions have been imposed.
The data requirements for colourants do not differ from those defined in 3-5.1, unless they are being used as hair dyes (see 3-5.5).

3-5.5 Hair dyes included in Annexes III and IV

Several hair dyes and hair dye components are present in Annexes III and IV to Dir. 76/768/EEC :

Annex IV contains a number of semi-permanent hair dyes, of which some azo-dyes are known to release one or more aromatic amines that have been classified as Carcinogen Category 2 through the Dangerous Substances legislation [1999/43/EC, 97/56/EC, 94/60/EC]. In 2002, the SCCNFP adopted an opinion discussing a large number of these components, concluding that the use of azo-dyes that release one or more carcinogenic aromatic amines, poses a potential risk to the health of the consumer [SCCNFP/0495/01].

Annex III, Part 1 contains a number of oxidative (permanent) hair dye components, such as diaminophenols, hydroquinone, 1-naphthol and resorcinol (ref. n° 10, 14, 16 and 22); while Annex III, Part 2, issued in 2002, provides provisional allowance for not less than 60 colouring agents used as oxidising and/or non-oxidising hair dye components [2002/34/EC]. In March 2003, the SCCNFP issued an opinion on the re-evaluation of 46 of these hair dyes [SCCNFP/0635/03].

A separate opinion on permanent hair dyes has been issued by the SCCNFP in June 2001 as a result of a study linking their use to an increased incidence of bladder cancer [Gago-Dominguez et al. 2001]. The SCCNFP concluded that further steps had to be taken to control the use of hair dye chemicals since the potential risks of using this category of substances clearly gave rise to concern [SCCNFP/0484/01].

The category of hair dyes most likely to cause adverse health effects is the oxidative hair dye two component system. One component contains the dye precursors (usually aromatic amines) and couplers (such as resorcinol- and phenol-derivatives) in an alkaline soap or syndet base, while the second component is a stabilised solution of hydrogen peroxide. The two components are mixed immediately prior to use [SCCNFP/0566/02].

The assessment of the genotoxicity/mutagenicity potential of oxidative hair dyes therefore is very complex, involving dye precursors, intermediates, reaction and final product(s) at the same time. As a consequence, the hazard of the oxidative hair dyes must 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.

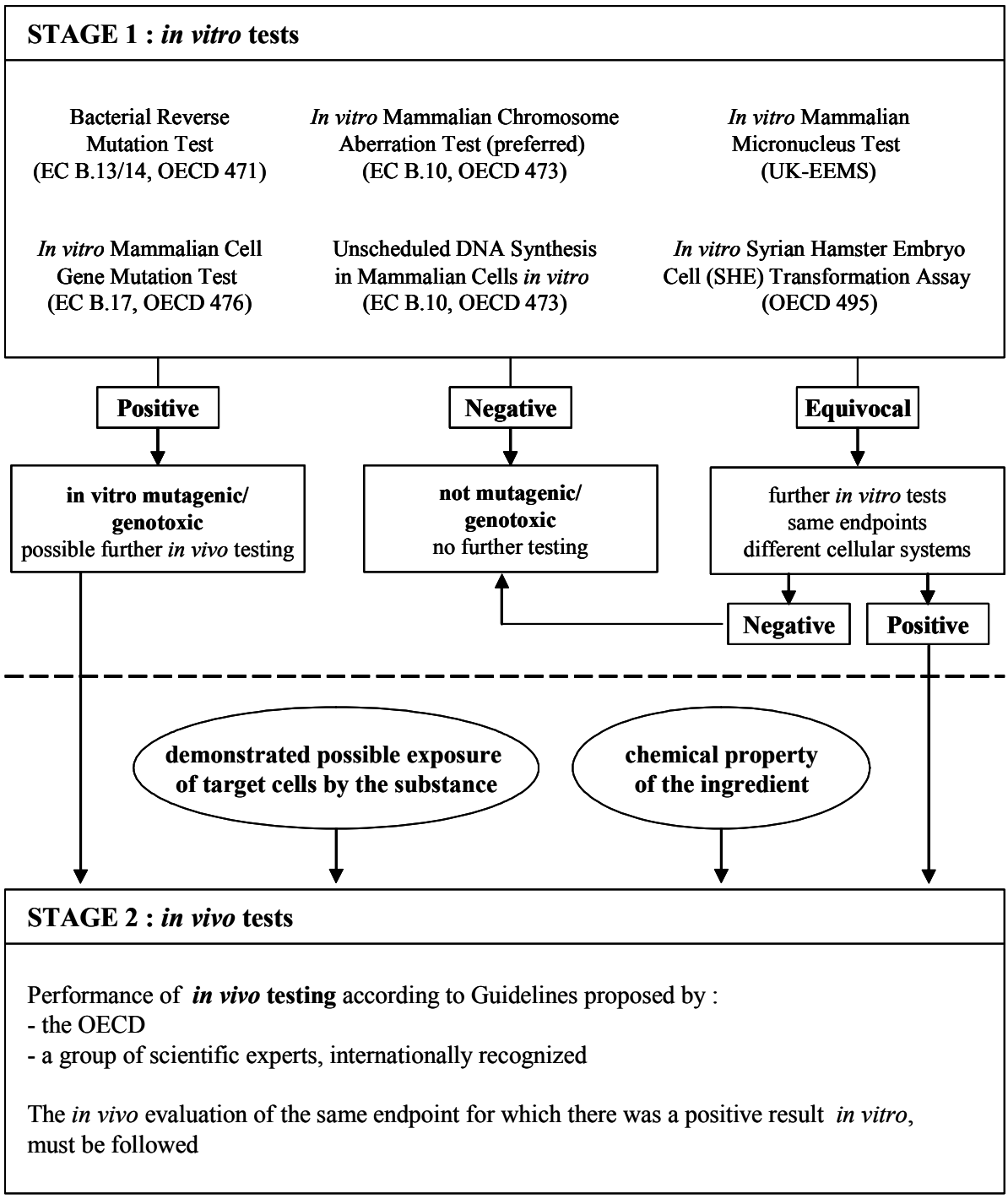

Fig. 2 : Schematic representation of the strategy proposed by the SCCNFP for the assessment of hair dyes [SCCNFP/0566/02 & SCCNFP/0720/03].

During its Plenary meeting of June 2002, the SCCNFP adopted a document describing a detailed testing strategy for testing hair dye cosmetic ingredients for their potential genotoxicity/mutagenicity [SCCNFP/0566/02]. This strategy was updated in June 2003 [SCCNFP/0720/03] and is summarized in Fig.2. It describes a flexible, stepwise *in vitro* strategy for hazard identification with regard to the mutagenic/genotoxic/carcinogenic potential of hair dyes, so that sufficient *in vitro* data may be obtained for adequate risk characterization. This strategy helps to minimize the use of animals, but it has not been validated yet.

Briefly summarized, this new approach imposes six *in vitro* tests in the first stage of mutagenicity/genotoxicity/carcinogenicity testing, which now comprises of :

1) Bacterial Reverse Mutation Test [EC B. 13/14, OECD 471];
2) *In vitro* Mammalian Chromosome Aberration Test [EC B.10, OECD 473]
3) *In vitro* Mammalian Cell Gene Mutation Test* [EC B.17, OECD 476];
4) Unscheduled DNA Synthesis in Mammalian Cells *in vitro* [EC B.18, OECD 482];
5) *In vitro* Mammalian Micronucleus Test [Miller et al. 1988, Parry 1997]
6) *In vitro* Syrian Hamster Embryo Cell (SHE) Transformation Assay [OECD 495]

These 6 assays cover the *in vitro* evaluation for mutagenicity/genotoxicity and do also give information the non-genotoxic/carcinogenic potential.

Stage 2 *in vivo* assays aim to ascertain whether a genotoxic/mutagenic effect shown *in vitro* may also occur in somatic cells under *in vivo* conditions. It is the applicant's responsibility to select the suitable stage 2 *in vivo* studies, taking into account the chemical structure of the hair dye, its metabolism, the expertise available and the results of earlier (*in vitro*) tests [SCCNFP/0720/03].
Moreover, special attention should be given to the isolation and identification of the reaction products during a simulation of the in-use conditions, to their exposure and bioavailability and to their submission to tiered genotoxicity/mutagenicity testing according to their relevance. In the case of relevant systemic exposure of reaction products observed during a simulated application, an animal model should be developed for evaluating the potential of the mutagenicity/genotoxicity/carcinogenicity of the reaction products in an *in vivo* situation.

Finally, a specific additional requirement for hair dyes recommended to DG Enterprise is the disposition of hair dye reference samples. It was proposed at the 7th Plenary Meeting of the SCCNFP of 17 February 1999 that industry should enclose a sample with each new submission to the Committee. Previously, in its meeting of 24 November 1998, the Working Party on Hair Dyes expressed the opinion that collection of samples was needed. Also, it was requested that stability information should be supplied.

* By preference the mouse lymphoma assay

3-5.6 Annex VI

Annex VI is a list of preservatives, including maximum allowed concentrations in finished products. The requirements for inclusion into this Annex are those as defined in 3-5.1.

3-5.7 Annex VII

Annex VII is a list of UV absorbing or UV reflecting substances with their maximum authorised concentrations in cosmetic products.

By their nature, all cosmetic ingredients used as sunscreens or UV absorbers are chemicals that either absorb or reflect UVA- and/or UVB-light. The range of the wavelengths that are absorbed by a given cosmetic ingredient is called its "absorption spectrum".
As a consequence of such light absorption, a chemical may undergo changes in its molecular configuration, or may be transformed into a different chemically reactive molecule. Hence there is a need to investigate specific phototoxic effects, such as photoirritancy, photosensitisation and photomutagenicity.
It is therefore evident that point 10. (Photo-induced toxicity) of the requirements tabled under 3-5.1 is crucial for the assessment of a possible inclusion of an ingredient in Annex VII.

All the studies relating to the phototoxic potential of an ingredient must be performed by applying the relevant UV light wavelengths derived from the absorption spectrum of the ingredient [SCCNFP/0633/02].

Photostability data under in use conditions should be provided.

a) in vitro photoclastogenicity/photomutagenicity

For the detection of photochemical clastogenicity/mutagenicity several well-established assays have been adopted to a combined treatment of chemicals with UV-VIS light including :

- bacterial and yeast mutation assays [Dean et al. 1991; Chetelat et al. 1993a and Averbech et al. 1979];
- tests for detecting clastogenicity [Gocke et al. 1998 and Chetelat et al. 1993b];
- tests for detecting gene mutations in mammalian cells [Pflaum et al. 1998; Chetelat et al. 1996];
- tests for detecting aneugenicity in mammalian cells *in vitro* [Kersten et al. 2002].

b) in vitro phototoxicity

Phototoxicity studies on cosmetic ingredients must be performed by applying the 3T3 NRU PT test, according to Guideline 432 adopted by the OECD in 2002 and incorporated as B.41 in Annex V to Dir. 67/548/EEC [2001/59/EC].

3-5.8 Requirements for partial evaluations

In some cases, either upon request of the SCCNFP or on a voluntary basis, industry provides additional data on substances that have been discussed in the past. An evaluation exclusively based on additional reports, together with summaries of earlier submissions may not be adequate to answer the question of the new risk.
As an example the re-evaluation study of Annex VI could be mentioned. This study was triggered by the fact that, besides **the intended use as a preservative**, some of the substances on Annex VI also include the phrase "for other uses" possibly in other (higher) concentrations than used for preservative purposes (these entries are marked by a (+)) [SCCNFP/0125/99].

Therefore, complete dossiers are required when a re-evaluation of only a part of a dossier is necessary [SCCNFP/0125/99].

3-6 BASIC REQUIREMENTS FOR COSMETIC INGREDIENTS (WHICH ARE EVALUATED BY INDIVIDUAL SAFETY ASSESSORS)

3-6.1 General toxicological requirements

Although the Mandate of the SCCNFP (described in 2-2) does not explicitly cover the safety assessment of ingredients not taken up in the Annexes to Dir. 76/768/EEC, some general considerations are provided in this chapter.

From the European legal point of view, many cosmetic ingredients simply have to comply with the requirements of the Dangerous Substances legislation [67/548/EEC and its amendments and adaptations to technical progress]. According to the latter for every newly notified chemical substance the required data package is triggered by the produced / EU imported annual volumes of that compound [92/32/EEC].
The simple fact that a substance will be used as a cosmetic ingredient, does not trigger any additional toxicological data requirement.

The toxicological requirements for dangerous substances produced /EU imported at levels between 100 kg and 1 tonne per year (a category to which several cosmetic ingredients belong), consist of :

- Acute toxicity (oral, dermal or inhalation)
- Skin and eye irritation
- Sensitisation
- Mutagenicity data

When higher amounts are produced/EU imported per year, a more extensive list of toxicological requirements is established [92/32/EEC].

A scientifically sound safety evaluation, based on less data than those mentioned above for the 100 kg - 1 tonne/year category, becomes quite impossible. Therefore, suppliers should be encouraged to deliver at least these data to all their customers in the cosmetic industry, in particular since many of these compounds are so-called "actives" and are not necessarily safe at all possible concentrations.

Therefore, it would be very useful if, in analogy with the ingredients taken up in the Annexes to Dir. 76/768/EEC, new information acquired by the suppliers, industry and/or other agencies, could be communicated to the customers in the cosmetic industry. When more elaborate data packages are available (e.g. high production volume chemicals), a large number of the general requirements described in 3-5.1 should become available.

In addition, the chemical nature of all cosmetic ingredients and their degree of purity, chemical and physical properties (as described in 3-3) should be ascertained. Upon request, the methods for identification and quantitative control should be made available to the relevant competent authorities of the Member States.

In the following paragraphs some general problems, caused by the nature and/or origin of the ingredients under consideration, are discussed.

3-6.2 Identification of mineral, animal, botanical and biotechnological ingredients

The nature and preparation of some ingredients may affect the type and amount of data necessary for their identification. The following points indicate the advised requirements for :

a) Complex ingredients of mineral origin

- starting material
- description of :
  - the preparation process : physical processing, chemical modifications, possible purification,
  - characteristic elements of the composition : characteristic components, toxic components (%).
- physical and chemical specifications
- microbiological quality
- preservatives added.

b) Complex ingredients of animal origin

- species (bovine, ovine, crustacean, human,…)
- organs, tissues, biological liquids (placenta, serum, cartilage,...)
- country of origin
- description of :
  - the preparation process : conditions of extraction (solvent, pH, temperature,…); type of hydrolysis (acidic, enzymatic,…); other chemical modifications; possible purification;
  - commercial form : powder, solution, suspension, freeze-dried,…
  - characteristic elements of the composition : characteristic amino acids, total nitrogen, polysaccharides, molecular mass,…
  - preservatives added
- physical and chemical specifications
- microbiological quality including relevant viral contamination
- additional external contamination
- preservatives added.

c) Complex ingredients of botanical origin

- common or usual names of the plant, alga or macroscopic fungus
- name of variety, species, genus, and family
- in case more than one variety of source of a given species is used, each should be specified
- organoleptic, macroscopic and microscopic evaluation
- morphological and anatomical description (including gender, if applicable) and a photograph of the plant or plant part, alga, or macroscopic fungus used
- natural habitat and geographical distribution of the plant, alga, or macroscopic fungus
- current sources of the plant, alga, or macroscopic fungus, including its geographical location and whether it is cultivated or harvested from the wild
- description of :
  - preparation process : collection, washing, drying, extraction, distillation, destructive distillation, possible purification, preservation procedures,…;
  - handling, transportation, storage;
  - commercial form : powder, solution, suspension,…;
  - characteristic elements of the composition : identification of characteristic components, toxic components (%);
- physical and chemical specifications
- microbiological quality including relevant fungi
- additional external contamination
- preservatives added.

d) Complex ingredients derived from biotechnology

For special biotechnologically derived ingredients, where a modified micro-organism or a potential toxic substance has not been fully removed, specific data must be available, which can comprise :

- description of organisms involved : donor organisms, recipient organisms, modified micro-organisms
- host pathogenicity
- toxicity, and when possible, identity of metabolites, toxins produced by the organisms
- fate of viable organisms in the environment-survival-potential for transfer of characteristics to e.g. natural bacteria
- physical and chemical specifications
- microbiological quality
- additional external contamination
- preservatives added.

3-6.3 Fragrance materials

Every fragrance compound should be accompanied by an adequate and duly signed certificate of conformity.

Although most fragrance suppliers deliver a standard certificate indicating the safe use of the fragrance compound within a range of concentrations per product type, it is the opinion of the SCCNFP that such certification should be systematically supplemented by :

- a semi-quantitative concentration of the ingredients in the fragrance compound (i.e., <0.1%; 0.1 to <1%, 1% to <5%, 5% to <10%, 10% to <20%, 20% and more) using the preferred terminology as indicated in Section II of the Inventory of Cosmetic Ingredients and the INCI name if available;
- for natural ingredients, there should be either
  1) an analysis of the composition of the batch of the natural ingredient, or
  2) an indication of the maximum levels of components which may be present in the natural ingredient, taking into account batch to batch variation;
- an indication of which of the ingredients have an established potential to cause contact sensitisation, phototoxicity, systemic toxicity etc., or are subject to restrictions either by industry guidelines, the Cosmetics Directive or by SCCNFP opinions [SCCNFP/0017/98, SCCNFP/0392/00, SCCNFP/0450/01, SCCNFP/0588/02]; a confirmation that any such restrictions have been conformed to;
- a clear indication of the types of cosmetic products in which the compound may be used and at what maximum concentration.

The above information should be available to the safety assessor of the finished cosmetic product. In the final risk evaluation, reference should be made to the semi-quantitative formulation of the fragrance compound and consideration taken as to the toxic potential of the ingredients considered singularly or in combination and with relevance to the finished cosmetic product considered as a whole.

3-6.4 Potential endocrine disruptors

In recent years, global concerns have been raised over the potential adverse effects that may result from exposure to chemicals that could have the potential to interfere with the endocrine system.
In 2000, DG Env (Directorate-General Environment) issued a document titled "Towards the establishment of a priority list of substances for further evaluation in their role in endocrine disruption" [M0355008/1786Q/10/11/00]. In this study, a working list of 564 substances was drawn up for which information on endocrine disrupting effects has been gathered. The study was carried out in four steps, being (1) a review of existing lists and other sources of information, (2) selection of highly persistent and/or high production volume (HPV) substances, (3) a preliminary evaluation of scientific evidence of endocrine disrupting effects and (4) a preliminary evaluation of exposure to humans and wildlife. Finally, a number of recommendations were formulated, including the need for standard tests and criteria for identifying endocrine disruption and the need for comparison of the endocrine disrupting effect evaluation with the concentrations at which toxic effects (mortality, reproduction, …) occur.

Two years later, the IPCS (International Programme on Chemical Safety) published a review on the same issue, taking together all publications, workshop / conference proceedings and expert committee evaluations on endocrine disruptors [Damstra et al. 2002]. This report states that concerns regarding exposure to these endocrine disruptors are primarily due to :

1) adverse effects observed in certain wildlife, fish and ecosystems,
2) the increased incidence of certain endocrine-related human diseases,
3) endocrine disruption resulting from exposure to certain environmental chemicals observed in laboratory experimental animals.

In 2001, cosmetic products were openly mentioned as potential endocrine disruptors. The reason was that in a scientific publication some *in vitro* and *in vivo* estrogenic effects were linked to a number of UV filters actually present in sun products [Schlumpf et al. 2001].
In June 2001, the SCCNFP issued an opinion on the matter and concluded that the study under discussion showed a number of important technical shortcomings.
One of the major points highlighted was that the *in vitro* potency of the UV filters studied, was not only importantly lower (1 million units) than the one observed for the positive control (17β-estradiol), but also very low in comparison with exposure to known "estrogenic" substances in food (flavonoids) and hormonal therapy (birth control pill, morning after pill, post-menopausal therapy).

After a critical analysis of all the available information, the SCCNFP came to the conclusion that, at present, the organic UV filters used in cosmetic sunscreen products allowed on the EU market, showed no estrogenic effects that could potentially affect human health [SCCNFP/0483/01].

3-6.5 BSE-issues

Commission Directive 97/1/EC, following an opinion issued by the SCC (02/10/1996), was at the origin of entry n° 419 of Annex II, stipulating that "bovine, ovine and caprine tissues and fluids from the encephalon, the spinal cord and the eyes, and ingredients derived therefrom" must not form part of the composition of cosmetic products.

The SCCNFP follows up closely the Commission Decisions regulating the use of material presenting risks as regards transmissible spongiform encephalopathies (TSEs), that update the list of tissues designated as Specified Risk Materials (SRMs) on the basis of the opinions issued by the Scientific Steering Committee (SSC). Although these Commission Decisions do not apply to cosmetic products, the Cosmetic Directive [76/768/EEC] should comply with their provisions [SCCNFP/0521/01].
Multiple SCCNFP opinions have been at the origin of several Commission Directives amending entry n°419 in order to align the list of prohibited animal materials to the acquired knowledge with respect to TSE.

The last adaptation to entry n° 419 in Annex II of Dir. 76/768/EEC was issued in January 2003 [2003/1/EC] and resulted in :

"**419.** From the date referred to in Article 22(1) of Regulation (EC) n° 999/2001 of the European Parliament and of the Council (OJ L147, 31.5.2001, p.1), the specified risk materials as designated in Annex V to that Regulation and ingredients derived therefrom.

Until that date, the specified risk materials as designated in Annex XI Part A to Regulation (EC) No 999/2001, and ingredients derived therefrom.

However, tallow derivatives may be used provided that the following methods have been used and strictly certified by the producer :

- transesterification or hydrolysis at at least 200°C and at an appropriate corresponding pressure for 20 minutes (glycerol and fatty acids and fatty acid esters)
- saponification with NaOH 12M (glycerol and soap) :
  - batch process : at 95°C for 3 hours
    or
  - continuous process : at 140°C, 2 bars (2000 hPa) for 8 minutes or equivalent conditions."

As indicated above, tallow derivatives of bovine origin are considered as an exception and are accepted as cosmetic ingredients provided they undergo a number of specific treatments. This exception was questioned by the SCCNFP in 2002 [SCCNFP/0612/02], but has been re-accepted in September 2003 [SCCNFP/0724/03].

At present, there is no evidence that TSE may be transmitted by topical exposure.

3-6.6 CMR-ingredients

Directive 2001/59/EC describes the criteria for classification of dangerous substances into categories 1, 2 or 3 for carcinogenicity, mutagenicity and reproduction (fertility and development) toxicity. To be classified into category 1 there must be sufficient evidence to establish a causal association between human exposure to a substance and the occurrence of the carcinogenic, mutagenic or reproduction toxic effect. Substances classified into category 2 should be regarded as if they are carcinogenic, mutagenic or reproduction toxic to man, while category 3 substances are defined as causing concern for man, but for which the available information is not adequate for making a satisfactory assessment [2001/59/EC].

As far as chemicals are concerned, Directive 94/60/EC clearly stipulates that substances appearing in Annex I to Council Directive 67/548/EEC and classified as carcinogens category 1 or 2, mutagens category 1 or 2, or toxic for reproduction category 1 or 2, may not be used in substances or preparations placed on the market **for use by the general public** in individual concentrations equal to or more than the concentration specified in Annex I to Directive 67/548/EEC, or the concentration specified in Annex I to Directive 1999/45/EC relating to the classification, packaging and labelling of dangerous preparations.

In its opinion of September 2001 [SCCNFP/0474/01], the SCCNFP proposed the prohibition of the intentional use in cosmetic products of substances classified according to Council Directive 67/548/EEC as CMR category 1 or 2 and substances with similar potentials. Substances classified according to Council Directive 67/548/EEC as CMR category 3 and substances with similar potentials should not intentionally be used in cosmetic products **unless** it can be demonstrated that their levels do not pose a threat to the health of the consumer. If a carcinogen, mutagen, or a substance toxic to reproduction is present in a cosmetic product from its presence in a natural ingredient, as an impurity, or because it is formed during the manufacture, it must be demonstrated that the product does not pose a threat to the health of the consumer [SCCNFP/0474/01].

The SCCNFP opinion on CMR substances has been translated into legislation through the "7th Amendment" [2003/15/EC], Art. 4b.

3-6.7 Hair dyes

Not every hair dye is included as a colourant in Annex IV to Directive 76/768/EEC. Nevertheless, the SCCNFP requirements for oxidative hair dyes as described under 3-5.5 must be considered for this category of cosmetic ingredients.
It is the opinion of the SCCNFP that a separate list of hair dyes should be established, for efficient regulation.

3-7 GENERAL PRINCIPLES FOR THE CALCULATION OF THE MARGIN OF SAFETY AND LIFETIME CANCER RISK FOR A COSMETIC INGREDIENT

3-7.1 Introduction : definitions

Dose : The amount of test substance administered.
Dose is expressed as weight (mg or g), as weight of test substance per unit weight of test animal (e.g. mg/kg body weight), as weight per unit of surface (e.g. mg/cm² of skin), or as constant dietary concentrations (ppm or mg/kg of food) [based on General Introduction: Part B, 96/54/EC].

Dosage : A general term comprising of dose, its frequency and duration. In the calculations of the Margin of Safety, dosage is expressed in mg/kg body weight/day [General Introduction: Part B, 96/54/EC].

NO(A)EL : The No Observed (Adverse) Effect Level is the outcome of long-term toxicity studies, such as 28-day, 90-day tests with rats, mice, rabbits or dogs, chronic toxicity tests, carcinogenicity tests, teratogenicity tests, reproduction toxicity tests, … It is the highest dosage for which no (adverse) effects can be observed [General Introduction: Part B, 96/54/EC].
In the calculation of the MoS, the lowest obtained NO(A)EL value is used, in order to consider the most sensitive species, as well as the relevant effect occurring at the lowest dosage possible.
The NO(A)EL should be expressed as mg/kg body weight/day.

SED : The Systemic Exposure Dosage () of a cosmetic ingredient is the amount expected to enter the blood stream (and therefore be systemically available) per kg body weight and per day. It is expressed in mg/kg body weight/day.
For this definition a mean human body weight of 60 kg is commonly accepted.
Since the majority of cosmetic products are applied topically, systemic availability will strongly depend on the dermal absorption of the compound. This can be determined according to the tests described under 3-4.4. Nevertheless, the results of these tests can be interpreted in two different ways (see 3-7.3 : dermal absorption issues).

3-7.2 The Margin of Safety

In risk characterisation, the last phase in the safety evaluation of a cosmetic ingredient, an uncertainty factor applies. For cosmetics, this factor is called the MoS. It is generally accepted that the MoS of a substance can be calculated by dividing its lowest NO(A)EL value by its possible SED.

$$MoS = \frac{NO(A)EL}{SED}$$

This MoS value is used to extrapolate from a group of test animals to an average human being, and subsequently from average humans to sensitive subpopulations (see Fig.3).

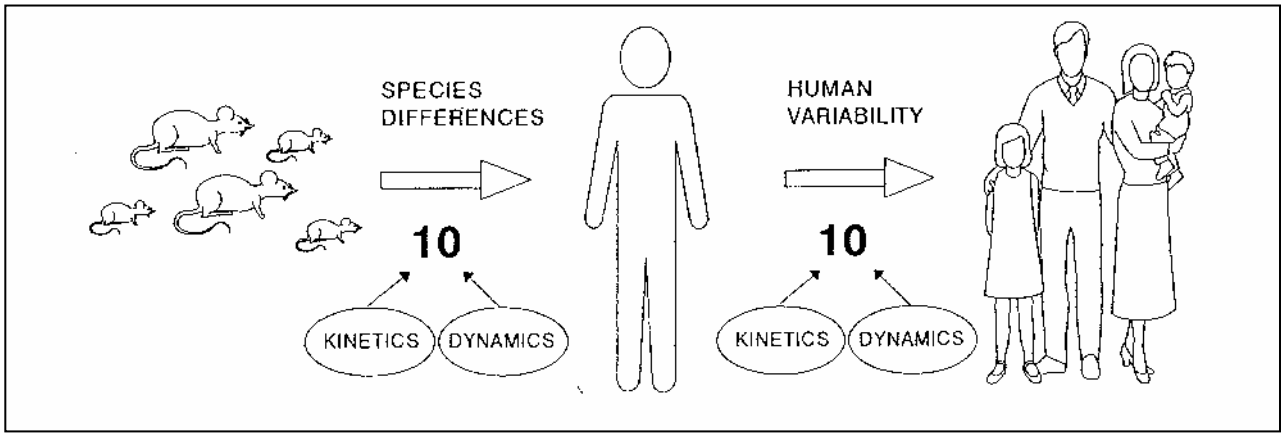

Fig.3 : Schematic representation of the extrapolation from animal to man [Renwick, 1998].

It is generally accepted that the MoS should at least be 100 to declare a substance safe for use.

As shown in Fig.3 this value consists of a factor 10 for the extrapolation from animal to man and another factor 10 taking into account the interindividual variations within the human population. These factors can be further subdivided as indicated in Fig.4 :

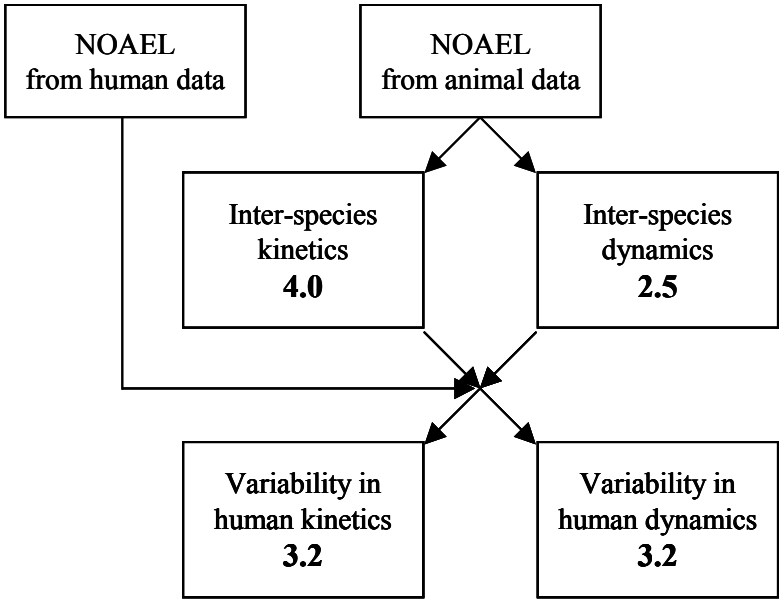

Fig.4 : Further subdivision of the Margin of Safety, taking into account kinetics and dynamics [based on WHO, 1994].

3-7.3 Dermal absorption issues in the calculation of the SED

Calculations of the SED should preferably be based on the absolute amount bioavailable (µg/cm²) after a certain time period, based on the highest anticipated concentration.
Calculations of the SED may also be based on the percentage dermally absorbed. In the latter case the resulting numbers depend on the dose applied on the skin. In this case, the concentrations tested should include the lowest concentration anticipated.

As proposed in OECD Draft Guideline 428 (Skin absorption: *in vitro* method), an application that mimics human exposure, normally 1-5 mg/cm² for a solid and up to 10 µl/cm² for liquids, should be used in *in vitro* tests.
Exceptions may exist, e.g. oxidative hair dyes, where 20 mg/cm² usually are applied for 30 minutes.

From the previous, it can be concluded that there are two ways of calculating the SED, depending on the way the dermal absorption of a compound is reported :

1) Dermal absorption of test substance reported in µg/cm² :

For calculating the SED, the skin surface envisaged to be treated with the finished cosmetic product containing the ingredient under study, has to be taken into account, as well as its frequency of application. All other variables should have been taken into consideration in the proper design of the dermal absorption study itself [SCCNFP/0750/03].

$$SED = \frac{\mathbf{DA_a}\ (\mu g/cm^2) \times 10^{-3} mg/\mu g \times \mathbf{SSA}\ (cm^2) \times \mathbf{F}\ (day^{-1})}{60\ kg}$$

With :
- SED (mg/kg bw/day) = Systemic Exposure Dosage
- DA_a (µg/cm²) = Dermal Absorption reported as amount/cm^2
- SSA (cm²) = Skin Surface Area expected to be treated with the finished cosmetic product (see section 6-2 for SSA values per product type)
- F (day^{-1}) = Frequency of application of the finished product
- 60 kg = default human body weight

2) Dermal absorption reported as a percentage of the substance applied :

It is clear that the percentage of dermal absorption will only be of value when calculated from *in vitro* studies with doses mimicking, but not exceeding the intended use conditions. Higher dose studies may result in an underestimation of the penetration.

The calculation of the SED will be as follows :

$$SED = \frac{\mathbf{A}\ (g/day) \times 1000 mg/g \times \mathbf{C}\ (\%)/100 \times \mathbf{DA_p}\ (\%)/100}{60\ kg}$$

With :
- SED (mg/kg bw/day) = Systemic Exposure Dosage
- A (g/day) = Amount of the cosmetic product applied daily : see the daily exposure values for different cosmetic product types (see 6-2)
- C (%) = the Concentration of the ingredient under study in the finished cosmetic product on the application site
- DA_p (%) = Dermal Absorption expressed as a percentage
- 60 kg = default human body weight

If the application mode is such that the number of applications differs from that mentioned in 6-2 for the intended product type, the SED will have to be adapted accordingly.

Finally, when considering dermal absorption, it is important to know whether the formulation can affect the bioavailability of one of its compounds. There are many penetration enhancers and excipients (such as liposomes) that are specifically added to a cosmetic formulation in order to facilitate dermal absorption of other compounds. It is clear that in such formulations, in the absence of further specific studies, 100% bioavailability of a particular ingredient will have to be assumed.

3-7.4 MoS for children

In its Plenary Meeting of February 2002, the SCCNFP issued an opinion on the calculation of the MoS for children. The question raised was whether it would be advisable to adjust the threshold factor of 100 for children by multiplying this factor by the difference in Skin Surface Area over Body Weight ratio (SSA/BW) between adults and children [SCCNFP/0557/02].

The difference between the SSA/BW ratio for children from 0 to 10 years is as follows :

2.3 fold at birth,
1.8 fold at 6 months,
1.6 fold at 12 months,
1.5 fold at 5 years,
1.3 fold at 10 years [Renwick 1998].

This implies that the mean average discrepancy between the SSA/BW children of 0 to 1 year of age and that of adults is only 1.9.
Looking back at Fig.4 under 3-7.2, this interindividual variation is already taken into account by the generally accepted threshold value of 100 for intact skin.
Therefore, and further based on the outcome of a symposium on toxicokinetics in children summarised by Renwick [1998], the SCCNFP concluded that, in general, there is no need for an additional uncertainty factor for children when **intact skin** is involved [SCCNFP/0557/02].

3-7.5 Lifetime cancer risk

In the case of non-threshold carcinogens it is assumed that there is no level of exposure that does not pose a small, but finite, probability of inducing cancer. Three methods for quantitative risk characterisation have been used or proposed by regulatory authorities in Europe and USA. The “Linearised Multistage Model” has been extensively used by the US EPA [1986]. The “LED_{10} method” has been proposed by the US EPA [1996b] and the "T_{25} method" has been used in Europe [Sanner et al., 2001]. The results obtained with these extrapolation methods are in most cases very similar.

Determination of the lifetime cancer risk is carried out in several distinct steps. After determination of an animal dose descriptor from the experimental data, the former is converted to a human dose descriptor. Subsequently, the lifetime cancer risk is determined by linear extrapolation to the actual exposure dose. Finally, a commentary statement is generated stating whether an overall evaluation of all data available indicates that the actual risk may be higher or lower than the calculated risk. The procedure is described in detail by Sanner et al. [2001].

The dose-descriptor T_{25} is defined as the chronic dose rate that will give 25% of the animal's tumours at a specific tissue site after correction for spontaneous incidence, within the standard life time of that species. The determination of T_{25} is described in details in EC 1999 and Dybing et al. [1997].
The animal dose descriptor (T_{25}) is converted to the human dose descriptor (HT_{25}) based on comparative metabolic rates, by using the following formula [Sanner et al. 2001] :

$$HT_{25} = \frac{T_{25}}{(\text{body weight}_{human}/\text{body weight}_{animal})^{0.25}}$$

Based on the daily lifetime dose, the lifetime cancer risk is calculated by linear extrapolation by use of the following formula:

$$\text{Lifetime cancer risk} = \frac{SED}{HT_{25} / 0.25}$$

SED represents the lifetime daily dose expressed in mg/kg bw/day.

Elements that affect risk estimates

Elements with a robust basis that can be expressed numerically should be incorporated in the lifetime cancer risks calculated above. Elements that cannot be expressed numerically should form the basis of a commentary statement.

Epidemiology : available epidemiological data, not sufficient for quantitative risk characterisation, nevertheless may be used for comparison with the risks derived from animal data.

Site/species/strain/gender activity : if the carcinogen is effective in multiple tissue sites and across species and genders, this may indicate that the risk may be higher than based on the calculation for one specific tumour type. If, on the other hand, the carcinogen is only active in a single specific tissue site in a single gender of a single species this may indicate that the risk may be lower than calculated.

Dose-response relationships : if the available data for the chosen tumor strongly suggest that linear extrapolation from the dose-descriptor value to some (very) low dose is not accurate and in fact indicate that the calculated risks are clearly under- or overestimating actual risks (i.e. the data indicate a supralinear or sublinear dose-response relationship for this part of the response curve, respectively), some qualitative or quantitative judgment can be made.

Chemical class : if the substance under consideration belongs to a chemical group with many carcinogens with T_{25}s clearly lower or higher than those of the carcinogen in question, and the confidence in the available data is low, the risk for this specific class member may be higher/lower than calculated.

Toxicokinetics : data on the relative bioavailability or target-dose of the carcinogen or its active metabolite in humans as compared to that in animals could indicate that the risk may be higher or lower than calculated from the animal data. A similar reasoning can be followed for toxicodynamic differences between humans and animals.

Additional elements relevant to risk evaluation : in cases that only one animal data-set is available for determination of the dose-descriptor or only data-sets from one animal species are available, there is greater uncertainty in the results than when data-sets for two species are available. Such cases could indicate that the risk might be higher than calculated from the animal data.

In cases where two or more data-sets are available for determination of the dose-descriptor and the other data-sets give significantly larger values for this parameter, this could indicate that the risk may be lower than calculated from the animal data.

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4. LISTS OF INGREDIENTS

4-1 INTRODUCTION

Five lists of cosmetic ingredients can be found as Annexes II, III, IV, VI and VII to Dir. 76/768/EEC. These annexes lay down clear limitations and requirements for the cosmetic ingredients concerned.

Another important list of cosmetic ingredients is the INCI (International Nomenclature of Cosmetic Ingredients) inventory [96/335/EC], identifying a large number of substances with their possible function(s) in finished cosmetic products and with reference to their possible restrictions.

Finally, this chapter briefly mentions Annex I to the Dangerous Substances legislation [67/548/EEC], since the "7th Amendment" of Dir. 76/768/EEC [2003/15/EC] directly refers to that list when excluding CMR Cat.1 & Cat.2 chemicals from cosmetic use.

It must be emphasized that none of these lists reflects the complete set of ingredients used in cosmetic products.

4-2 ANNEXES II, III, IV, VI AND VII TO THE COSMETIC PRODUCTS DIRECTIVE

The Cosmetic Products Directive [76/768/EEC and its adaptations to technical progress] defines the following Annexes :

Annex II : a list containing substances that must not form part of the composition of cosmetic products.

Annex III : a list of substances that are allowed to be used in cosmetic products, but only subject to the restrictions and conditions laid down.

Annex IV : a list of colourants allowed for use in cosmetic products in one of the 4 following fields of application :
1) all cosmetic products,
2) cosmetic products that are not applied in the vicinity of the eyes,
3) cosmetics that have no contact with mucous membranes
4) cosmetics that come only briefly in contact with the skin.

Annex VI : a list of preservatives that cosmetic products may contain. Preservatives are substances that may be added to cosmetic products for the primary purpose of inhibiting the development of micro-organisms in such products. Some of the preservatives in Annex VI are marked with a "(+)", which means that they may also be added to cosmetics in other concentrations for other scientific purposes apparent from the presentation of the products, e.g. as deodorants in soaps and anti-dandruff agents in shampoos.

Annex VII : a list of UV filters that cosmetic products may contain. For the purpose of Dir. 76/768/EEC, UV filters are substances that, contained in cosmetic sunscreen products, are specifically intended to filter certain UV rays in order to protect the skin from certain harmful effects of these rays.

Other UV filters, used in cosmetic products solely for the purpose of protecting the product against UV rays, are not included in Annex VII. They have not been submitted to and discussed by the SCCNFP.

Annexes III, IV, VI and VII are subdivided in 2 parts, Part 1 being the major list of "definitively" allowed ingredients, while Part 2 is a list of provisionally allowed substances. In time, every substance appearing on Part 2 of an Annex will either be added to Annex II (forbidden substance), taken up in Part 1 of the respective Annex ("definitively" allowed), or simply completely deleted from the Annex.

The applicability of the Annexes for the Member States is given in Articles 4.1 and 5 of Directive 76/768/EEC and it amendments :

	Art. 4.1 : Member States shall **prohibit** marketing of cosmetics containing :	**Art. 5 :** Member States shall **allow** marketing of cosmetics containing :
Annex II	substances listed in Annex II	
Annex III	substances listed in the first part of Annex III, beyond the limits and outside the conditions laid down	the substances listed in Annex III, Part 2, within the limits and under the conditions laid down, up to the dates in column (g) of that Annex
Annex IV	colouring agents : - other than those listed in Annex IV, Part 1 (exception for colouring agents intended solely to colour hair) - listed in Annex IV, Part 1, used outside the conditions laid down (exception for colouring agents intended solely to colour hair)	colouring agents listed in Annex IV, Part 2, within the limits and under the conditions laid down, until the admission dates given in that Annex
Annex VI	preservatives : - other than those listed in Annex VI, Part 1 - listed in Annex VI, Part 1, beyond the limits and outside the conditions laid down, unless other concentrations are used for specific purposes apparent from the presentation of the product	the preservatives listed in Annex VI, Part 2, within the limits and under the conditions laid down, until the dates given in column (f) of that Annex. However, some of these substances may be used in other concentrations for specific purposes apparent from the presentation of the product
Annex VII	UV filters : - other than those listed in Annex VII, Part 1 - listed in Annex VII, Part 1, beyond the limits and outside the conditions laid down therein	the UV filters listed in Annex VII, Part 2, within the limits and under the conditions laid down, until the dates given in column (f) of that Annex

4-3 THE INTERNATIONAL NOMENCLATURE OF COSMETIC INGREDIENTS (INCI)

Article 5a of Dir. 76/768/EEC states that the Commission shall compile an inventory of ingredients employed in cosmetic products [93/35/EEC].

On 8 May 1996, the European Commission established an Inventory and a common nomenclature of the ingredients employed in cosmetic products [96/335/EC].

This list was subdivided into 2 sections :

Section I : Inventory of ingredients employed in cosmetic products

Section II : Perfume and aromatic raw materials

The Inventory is indicative and does not constitute a list of substances authorized for use in cosmetic products. If an INCI name is available, it is to be used on the packaging and labelling, but the absence of an INCI name on the Inventory does not automatically exclude the use of the ingredient under consideration.
An entry in the Inventory provides identification of that particular ingredient through the following parameters :

- Common name : INCI; but botanicals get their systemic [Linné] Latin names and colours a colour index [CI] number
- Chemical name
- Chemical Abstract Service (CAS) number
- Cosmetic, Toiletry and Fragrance Association (CTFA) name
- European Pharmacopoeia (Ph. Eur.) name
- International Non-proprietary Name (INN) name, recommended by WHO
- International Union of Pure and Applied Chemistry (IUPAC) name
- European INventory of Existing commercial Chemical Substances (EINECS) number
- European List of Notified Chemical Substances (ELINCS) number

In 1998 the European Commission issued a Mandate [DG24/XXIV/1891/98], indicating that the SCCNFP shall act as a resource of scientific expertise to the European Commission, in terms of advising on the :

- medical and professional expectations and requirements of the Inventory,
- scientific accuracy and validity of proposed entries,
- outstanding needs of the existing text / proposed improvements in subsequent updates.

After a collaboration with the JRC (Joint Research Centre) of the Commission, experts from European Industry and Colipa (the European Cosmetic Toiletry and Perfumery Association), the SCCNFP issued a Status Report on the Inventory [SCCNFP/0098/99]. In this report, 6 priorities were identified for a first update of the INCI list :

1) To accomplish the principle : each INCI name should refer to only one specific ingredient.
2) To correct the INCI names of Ethylhexyl derivatives and to adopt a final decision on Ampho-derivatives.
3) To identify botanical entries with greater transparency.
4) To solve problems on chemical identification associated to polymers.
5) To solve the problem of hair dyes / cosmetic colourants with respect to Colour Index (CI) identification and restrictions.
6) To improve the description of the functions of the ingredients.

Having taken into account this list of priorities, the SCCNFP published in June 2000 "The 1st Revision and Update of Section I of the Inventory of ingredients employed in cosmetics" [SCCNFP/0299/00]. This update contains many improvements to the original edition of Section I, including 1466 new and 843 modified INCI names, as well as a number of necessary recommendations for future updating of the inventory.

In October 2000, "The 1st Update of the Inventory of ingredients employed in cosmetic products : Section II : Perfume and aromatic raw materials" was issued [SCCNFP/0389/00]. Again, many improvements were introduced (e.g. 650 new entries of botanicals) and recommendations for future updates were added.

Although the Inventory should be periodically updated by the Commission [93/35/EEC, Art. 5.a.3], there has been no official publication by the Commission since 1996.

4-4 ANNEX I TO THE DANGEROUS SUBSTANCES DIRECTIVE

Annex I to Dir. 67/548/EEC gives the list of dangerous substances classified in accordance with the provisions stated in Annex VI to that same Directive laying down the general classification and labelling requirements for dangerous substances and preparations in the EU [2001/59/EC]. Annex I is updated on a regular basis and contains a number of chemicals that can be found in the composition of certain cosmetic products. It has become of particular importance with the introduction of Art. 4b in the "Seventh Amendment" [2003/15/EC] to the Cosmetic Products Directive :

Art. 4b : *The use in cosmetic products of substances classified as carcinogenic, mutagenic or toxic for reproduction, of category 1, 2 and 3, under Annex I to Directive 67/548/EEC shall be prohibited. ... A substance classified in category 3 may be used in cosmetics if the substance has been evaluated by the SCCNFP and found acceptable for use in cosmetic products.*

Even though the majority of chemical substances occurring on Annex I to Dir. 67/548/EEC have no use in cosmetics, this list must be consulted in order to identify classified CMR Cat.1, 2 or 3 ingredients.

4-5 REFERENCES

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76/768/EEC - Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.
Official Journal L 262, 27/09/1976 p.169.

93/35/EEC - Council Directive 93/35/EEC of 14 June 1993 amending for the sixth time Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products.
Official Journal L 151, 23/06/1993 p.32.

96/335/EC - Commission Decision of 8 May 1996 establishing an inventory and a common nomenclature of ingredients employed in cosmetic products.
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SCCNFP/0098/99, Final : Status report on the inventory of cosmetic ingredients, *approved by the plenary session of the SCCNFP on 17 February 1999*.

SCCNFP/0299/00, Final : Opinion on the 1st update of the inventory of ingredients employed in cosmetic products (Section I), *adopted by the SCCNFP during the 13th plenary session of 28 June 2000*.

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1. TERMS OF REFERENCE

1.1 Context of the question

The adaptation to technical progress of the Annexes to Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.

Request for inclusion of ….. in Annex …, part 1 – List of ……. which Cosmetic Products may contain – to Council Directive 76/768/EEC.

1.2 Request to the SCCNFP

The SCCNFP is requested to answer the following questions :

* Is ……. safe for use in cosmetic products?
* Does the SCCNFP propose any restrictions or conditions for its use in cosmetic products?

1.3 Statement on the toxicological evaluation

The SCCNFP is the scientific advisory body to the European Commission in matters of consumer protection with respect to cosmetics and non-food products intended for consumers.

The Commission's general policy regarding research on animals supports the development of alternative methods to replace or to reduce animal testing when possible. In this context, the SCCNFP has a specific working group on alternatives to animal testing which, in co-operation with other Commission services such as ECVAM (European Centre for Validation of Alternative Methods), evaluates these methods.

SCCNFP opinions include evaluations of experiments using laboratory animals; such tests are conducted in accordance with all legal provisions and preferably under chemical law regulations. Only in cases where no alternative method is available will such tests be evaluated and the resulting data accepted, in order to meet the fundamental requirements of the protection of consumer health.

2. CHEMICAL AND PHYSICAL SPECIFICATIONS

2.1 Chemical identity

2.1.1 Primary name and/or INCI name

Ref. :

2.1.2 Chemical names

Ref. :

2.1.3 Trade names and abbreviations

Ref. :

2.1.4 CAS no.

Ref. :

2.1.5 Structural formula

Ref. :

2.1.6 Empirical formula

Ref. :

2.2 Physical form

Ref. :

2.3 Molecular weight

Ref. :

2.4 Purity, composition and substance codes

Ref. :

2.5 Impurities / accompanying contaminants

Ref. :

2.6 Solubility

Ref. :

2.7 Partition coefficient (Log P_{ow})

Ref. :

2.8 Additional physical and chemical specifications

Where relevant :

- organoleptic properties (colour, odour, taste if relevant)
- flash point
- vapour pressure
- boiling point
- melting point
- density
- viscosity
- pKa
- UV light absorption spectrum
- …

Ref. :

3. FUNCTION AND USES

Ref. :

4. TOXICOLOGICAL EVALUATION

4.1 Acute toxicity

4.1.1 Acute oral toxicity

Ref. :

4.1.2 Acute dermal toxicity

Ref. :

4.1.3 Acute inhalation toxicity

Ref. :

4.2 Irritation and corrosivity

4.2.1 Skin irritation

Ref. :

4.2.2 Mucous membrane irritation

Ref. :

4.3 Skin sensitisation

Ref. :

4.4 Dermal / percutaneous absorption

Ref. :

4.5 Repeated dose toxicity

4.5.1 Repeated dose (28 days) oral / dermal / inhalation toxicity

Ref. :

4.5.2 Sub-chronic (90 days) oral / dermal / inhalation toxicity

Ref. :

4.5.3 Chronic (> 12 months) toxicity

Ref. :

4.6 Mutagenicity / genotoxicity

Ref. :

4.7 Carcinogenicity

Ref. :

4.8 Reproductive toxicity

4.8.1 2-Generation reproduction toxicity

Ref. :

4.8.2 Teratogenicity

Ref. :

4.9 Toxicokinetics

Ref. :

4.10 Photo-induced toxicity

4.10.1 Phototoxicity/photoirritation and photosensitisation

Ref. :

4.10.2 Phototoxicity / photomutagenicity / photoclastogenicity

Ref. :

4.11 Human data

Ref. :

4.12 Special investigations

Ref. :

4.13 Safety evaluation (including calculation of the MoS)

Ref. :

4.14 Conclusions

4.15 References

5. OPINION OF THE SCCNFP

6. OTHER CONSIDERATIONS (IF ANY)

7. MINORITY OPINIONS (IF ANY)

6. SAFETY EVALUATION OF FINISHED COSMETIC PRODUCTS

6-1 INTRODUCTION

In accordance with the Sixth [93/35/EEC] and "Seventh" [2003/15/EC] Amendment to Council Directive 76/768/EEC, a technical information file (TIF) must be kept available by the manufacturer or importer of each cosmetic product within the EU and made accessible to the competent authorities of the Member States on demand. In particular, the TIF of a given cosmetic product must contain its safety evaluation, made by a safety assessor, with the competence as required in Art 7a(d) and being responsible for it. The safety evaluation of the finished product is based upon the toxicological profile of the ingredients, their chemical structure and their exposure level.

Although the Mandate of the SCCNFP (see 2-2) does not explicitly cover the safety evaluation of finished cosmetic products, some practical guidance is provided in this chapter. However, it must be emphasised that it remains the responsibility of the safety assessor to justify whether enough information on the ingredients, the finished product and exposure is available or whether additional data are needed to evaluate the cosmetic product under consideration. Therefore, the guidance hereafter should not be used as a checklist but rather as a tool to be adapted on a case by case basis when evaluating the safety of a finished cosmetic product [SCCNFP/0321/00].

6-2 CATEGORIES OF COSMETIC PRODUCTS AND EXPOSURE LEVELS IN USE

The evaluation of the safety of a cosmetic product is not only based on its intrinsic toxicological properties, but also on how it will be used. Since cosmetic products cover a wide range of product types, many exposure scenarios can be described, e.g. :

- soaps are applied in dilute form and, although the area of application may be extensive, the product is rapidly washed off,
- products used on the lips and mouth will be ingested to some extent,
- cosmetics used around the eyes and genital regions may come into contact with the conjunctiva or mucosa, respectively, potentially resulting in reactions due to the thin epithelial lining of these areas,
- body lotions or body creams may be applied over a large surface of the body and the ingredients, often at appreciable concentrations, may remain in contact with the skin for several hours,
- sunscreens, due to their extensive skin contact, combined with direct exposure to UV radiation for prolonged periods, require a distinct type of safety evaluation (see also section 3-5.7),
- permanent hair dyes undergo oxidative reactions on the hair and the intermediates and final products formed come into contact with the skin.

Every specific exposure scenario will be linked to a certain amount of substance that may be ingested or absorbed through the skin or mucous membranes. Translated into a daily amount per kg body weight, it is considered the Systemic Exposure Dosage (SED) of the finished cosmetic product.

It is clear that in use exposure levels can only be obtained on a case-by-case basis for cosmetic products, taking into consideration at least the following factors :

- class of cosmetic product(s) in which the ingredient may be used,
- method of application: rubbed-on, sprayed, applied and washed off, etc.,
- concentration of the ingredient in the finished cosmetic product,
- quantity of product used at each application,
- frequency of application,
- total area of skin contact,
- site of contact (e.g., mucous membrane, sunburnt skin),
- duration of contact (e.g., rinse-off products),
- foreseeable misuse which may increase exposure,
- consumer target group (e.g., children, people with "sensitive skin"),
- quantity likely to enter the body,
- projected number of consumers,
- application on skin areas exposed to sunlight.

Moreover, the relevant exposure depends upon the toxicological effects under consideration. For example, for skin irritation or phototoxicity the exposure per unit area of skin is important, while for systemic toxicity the exposure per unit of body weight is of more significance.
The possibility of secondary exposure by routes other than those resulting from direct application also should be considered (e.g. inhalation of hairsprays, ingestion of lip products, …).
Finally, the usage of cosmetic products may depend on some factors that will vary over time, such as age group, seasonal variations, local habits, fashion, trends, disposable income, product innovation, etc.

As previously mentioned, exposure assessment will among others result in the determination of the Systemic Exposure Dosage (SED), an important parameter for calculating the Margin of Safety (MoS) of ingredients in a finished cosmetic product [MoS = NO(A)EL / SED].
However, depending on the case whether the dermal absorption is reported in µg/cm² or as a percentage of the substance applied, different exposure parameters must be known in order to be able to calculate the actual SED :

1) Dermal absorption of test substance reported in µg/cm² :

$$SED = \frac{\mathbf{DA_a}\ (\mu g/cm^2) \times 10^{-3} mg/\mu g \times \mathbf{SSA}\ (cm^2) \times \mathbf{F}\ (day^{-1})}{60\ kg}$$

With :
- SED (mg/kg bw/day) = Systemic Exposure Dosage
- DA_a (µg/cm²) = Dermal Absorption reported as amount/cm²
- SSA (cm²) = Skin Surface Area expected to be treated with the finished cosmetic product
- F (day^{-1}) = Frequency of application of the finished product
- 60 kg = default human body weight

The use of this expression implies that the **skin surface area (SSA)** envisaged to be treated with the finished cosmetic product containing the ingredient under study, has to be known, as well as the **frequency of application (F)** of the finished product.
The first three columns of Table 1 are extracted from a Dutch study on cosmetic exposure assessment performed by the RIVM (RijksInstituut voor Volksgezondheid & Milieu) [Bremmer et al. 2003] and indicate exposed skin surface areas per cosmetic product type. The last column of the same table reflects the equivalent skin surface areas, based on US EPA [US EPA 1997] default values for skin surface areas of relevant parts of the human body. Some of the cells of this last column have been left blank, because :

- detailed parameters for some product types are missing [Bremmer et al. 2003],
- for some of the parameters described in the RIVM study [Bremmer et al. 2003], no equivalent values could be found in the USA EPA [1997] study.

Table 1 : Mean exposed skin surface area per product type
[Bremmer et al. 2003; US EPA 1997]

Product type	**Skin surface area involved (RIVM)**		**EPA equivalent surface area (cm²)**
	Surface area (cm²)	**Parameters**	
Hair care			
Shampoo	1440	area hands + 1/2 area head	1430
Hair conditioner	1440	area hands + 1/2 area head	1430
Hair spray	565	1/2 area head female	555
Hair styling gel	1010	1/2 area hands, 1/2 area head	1010
Hair styling mousse	1010	1/2 area hands, 1/2 area head	1010
Hair dye spray	580	1/2 area head	590
Oxidation or permanent hair dyes	580	1/2 area head	590
Hair bleach	580	1/2 area head	590
Hair permanent lotion	580	1/2 area head	590
Hair fixing lotion	580	1/2 area head	590
Bathing, showering			
Hand wash soap liquid	860	area hands	840
Hand wash soap solid	860	area hands	840
Showering soap liquid	17500	total body area	19400
Showering soap solid	17500	total body area	19400
Bath foam	16340	area body + area head	
Bath salt	16340	area body + area head	
Bath oil	16340	area body + area head	

Product type	Skin surface area involved (RIVM)		EPA equivalent surface area (cm²)
	Surface area (cm²)	Parameters	
Skin care			
Face cream	565	1/2 area head female	555
Body lotion	15670	area body + area head female	
Hand cream	860	area hands	840
Peeling / scrubbing gel	565	1/2 area head female	555
Face pack	565	1/2 area head female	555
Body pack	15670	area body + area head female	
Skin whitening cream	565	1/2 area head female	555
Make-up and nail care			
Facial make-up	565	1/2 area head female	555
Facial cleanser	565	1/2 area head female	555
Eye shadow	24		
Mascara	1.6		
Eyeliner	3.2		
Eye make-up remover	50		
Nail polish	4		
Nail polish remover	11		
Deodorant			
Deodorant stick / roller	100		
Deodorant spray	100		
Foot care			
Foot cream antiperspirant	1170	area feet	1120
Foot cream anti-fungal	1170	area feet	1120
Fragrances			
Eau de toilette spray	200		
Perfume spray	100		
Men's cosmetics			
Shaving cream	305	1/4 area head male	325
Aftershave	775	1/4 area head male + 1/2 area hands male	820
Sun care cosmetics			
Sunscreen lotion	17500	total body area	19400
Sunscreen cream	17500	total body area	19400

Product type	Skin surface area involved (RIVM)		EPA equivalent surface area (cm²)
	Surface area (cm²)	Parameters	
Baby care			
Baby cream	189		
Baby oil	189		
Baby powder	189		
Miscellaneous			
Depilatory cream	5530	area female legs	5460
Essential oil massage	16340	area body + area head	
Essential oil bath	16340	area body + area head	
Child face paint	475	1/2 area child head (4.5 yrs old)	496
Adult face paint	580	1/2 area male head	650

2) Dermal absorption reported as a percentage of the substance applied :

$$SED = \frac{\mathbf{A}\ (g/day) \times 1000 mg/g \times \mathbf{C}\ (\%)/100 \times \mathbf{DA_p}\ (\%)/100}{60\ kg}$$

With : SED (mg/kg bw/day) = Systemic Exposure Dosage
A (g/day) = Amount of the cosmetic product applied daily
C (%) = the Concentration of the ingredient under study in the finished cosmetic product on the application site
DA_p (%) = Dermal Absorption expressed as a percentage
60 kg = default human body weight

In this case the **daily Amount of formulation applied (A)** under intended in use conditions has to be known.

Displayed on the next page, table 2 summarizes existing Colipa cosmetic exposure data [SCCNFP/0321/02].

Robust exposure data, based on current use patterns relevant to the European consumer, are awaited and will replace the data in table 2.

Table 2 : Calculation of the daily exposure to cosmetics using Colipa data [SCCNFP/0321/02].

Product type	Amount of substance applied	Frequency of application	Retention factor††††	Daily exposure calculated
Hair care				
Shampoo	8.0 g	1 / day	0.01	0.08 g/day
Hair conditioner	14.0 g	0.28 / day	0.01	0.04 g/day
Hair styling products	5.0 g	2 / day	0.1	1.00 g/day
Oxidation or permanent hair dyes	100 ml	1 / month (30 min.)	0.1	Not calculated×
Semi-permanent hair dyes (and lotions)	35 ml	1 / week (20 min.)	0.1	Not calculated×
Bathing, showering				
Shower gel	5.0 g	2 / day	0.01	0.10 g/day
Skin care				
Face cream	0.8 g	2 / day	1.0	1.6 g/day
General purpose cream	1.2 g	2 / day	1.0	2.4 g/day
Body lotion	8.0 g	1 / day	1.0	8.0 g/day
Make-up and nail care				
Make-up remover	2.5 g	2 / day	0.1	0.5 g/day
Eye make-up	0.01 g	2 / day	1.0	0.02 g/day
Mascara	0.025 g	1 / day	1.0	0.025 g/day
Eyeliner	0.005 g	1 / day	1.0	0.005 g/day
Lipstick, lip salve	0.01 g	4 / day	1.0	0.04 g/day
Deodorant				
Deodorant stick / roller	0.5 g	1.0 / day	1.0	0.50 g/day
Oral hygiene				
Toothpaste (adult)	1.4 g	2.0 / day	0.17	0.48 g/day
Mouthwash	10.0 g	3.0 / day	0.10	3.0 g/day
Sun care cosmetics				
Sunscreen lotion				18.0 g/day

†††† The retention factor was introduced by the SCCNFP to take into account rinsing off and dilution of finished products by application on wet skin or hair (e.g. shower gels, shampoos, …) [SCCNFP/0321/00]

× Daily exposure value not calculated due to the low frequency of exposure

In the specific case of preservatives, individual product type exposure values as mentioned in table 2 might not reflect the overall exposure to these compounds, since there is a clear possibility that a certain preservative will not only be used in the finished cosmetic product under consideration, but also in a number of other cosmetics used by the same consumer. Therefore, the SCCNFP calculated a **global daily exposure value** for all cosmetic products that one person may daily apply on the skin. In a worst-case scenario, considering the consumer would use a set of cosmetic products containing the same preservative, the SCCNFP-value of **17.79 g/day** will have to be used in the calculation of the MoS [SCCNFP/0321/00].

6-3 GUIDELINES FOR THE SAFETY EVALUATION OF FINISHED COSMETIC PRODUCTS

6-3.1 Introduction

Each cosmetic product is considered as an individual combination of cosmetic ingredients. It is generally accepted that the safety evaluation can be done by ascertaining the toxicity of its ingredients [93/35/EEC & 2003/15/EC] on the condition that the information on the most relevant toxicological endpoints of its constituent ingredients is available. In some cases, however, additional information on the finished product is needed in the interest of a better safety assessment. Examples are cosmetics for specific target consumers groups (babies, sensitive skin, etc.), the presence of certain ingredients that increase skin penetration and/or skin irritancy (penetration enhancers, organic solvents, acidic components, etc.), the presence of a chemical reaction between individual ingredients rendering the formation of a new substance of toxicological significance highly probable, the presence of a specific galenic form (liposomes and other vesicular forms, etc.), when the potential toxicity of a particular ingredient is claimed to be decreased, etc.

When, after an in-depth evaluation of the safety of the final product, the safety assessor does not expect it to cause any adverse effect under foreseeable conditions of use, it is recommended to undertake compatibility testing on a number of human volunteers before the product is finally marketed [SCCNFP/0068/98].

6-3.2 Toxicological profile of the ingredients

During the safety evaluation of a finished cosmetic product, the available toxicological data for all ingredients should be taken into consideration by the safety assessor. The data sources used should be clearly indicated and may consist of one or more of the following possibilities :

- *in vivo* tests using experimental animals,
- *in vitro* tests using validated or valid alternative methods,
- human data from clinical observations and compatibility tests in human volunteers,
- data from data banks, published literature, "in house" experience and data obtained from raw data suppliers, including QSAR structural alerts,
- relevant data on analogous compounds,

The general toxicological requirements for cosmetic ingredients have been described in detail in chapter 3 of this document.

For cosmetic products, focus lays in particular on local toxicity evaluation being skin and eye irritation, skin sensitisation, and in the case of UV absorption photo-induced toxicity. In case of significant dermal /percutaneous absorption, systemic effects will also to be examined in detail. When certain test results are not available, a scientific justification should be included.

It is essential to mention here that for each ingredient the toxicological data given should be derived from tests with the same substance as that used in the finished cosmetic product (same degree of purity, same impurity profile, same additives, …).

6-3.3 Stability and physical and chemical characteristics of the finished cosmetic product

The physical stability of the finished product should be established, ensuring that no changes in physical state of the finished product (e.g. coalescence of emulsions, phase separation, crystallisation or precipitation of ingredients, colour changes, …) occur during transport, storage or handling of the product. Indeed, exposure to changing temperatures, humidity, UV light, mechanical stress … could reduce the intended quality of the product and the safety for the consumer.
Relevant stability tests, adapted to the type of cosmetic product and its intended use, should be carried out. To make sure that no stability problems are induced by the type of container and packaging used, physical stability tests are currently carried out with inert containers and those intended to be used on the market.

Relevant physical and chemical parameters should be controlled for each batch of the finished product coming on the market. General parameters could be:

- physical state,
- type of preparation (emulsion o/w or w/o, suspension, lotion, powder, aerosol, …),
- organoleptic properties (colour, odour, whenever relevant),
- pH (at ..°C) for aqueous preparations,
- viscosity (at ..°C) for liquid forms,
- other according to specific needs.

The criteria and methods used, and the results obtained per batch should be specified.

6-3.4 Evaluation of the safety of the finished product

The scientific reasoning by the safety assessor must be clearly described in the safety evaluation report of the finished product. This means that all toxicological data available on the individual ingredients and the end product (favourable and unfavourable), all chemical and/or biological interactions and human exposure via intended and likely routes must be taken into account. Whenever a NO(A)EL value is available for a specific ingredient, its Margin of Safety (MoS) should be calculated and taken into account.

The conclusions made by the safety assessor must be well-argued and the inclusion in the formulation of particular ingredients of special concern must receive special attention (e.g. perfume, UV filters, hair dyes, etc.). The safety assessor may accept, reject, or accept under specific conditions the formulation under consideration. Recommendations by the safety assessor, which are relevant for the safety-in-use of the product, must be followed up by the responsible manufacturer or EU importer.

The curriculum vitae of the safety assessor must be included in the dossier. The safety assessor may be employed by the manufacturer or may be an external consultant. No connection should exist with production or marketing. The safety assessor must provide evidence of having relevant experience in toxicology, as well as a controlled independence in matters of product related decision.

Finally, the safety of the product should be reviewed on a regular basis. To that end, undesirable effects on human health during in market use of the product should be filed (complaints during normal and improper use, and the follow-up done) and taken into account in the next safety assessment of the product.

As indicated before (see Fig.1 under section 3-2), the safety evaluation of finished cosmetic products is not the responsibility of the SCCNFP.

6-4 GUIDELINES ON MICROBIOLOGICAL QUALITY OF THE FINISHED COSMETIC PRODUCT

6-4.1 Preamble

Skin and mucous membranes are protected from microbial attack by a natural mechanical barrier and various defence mechanisms. However, the protective integument may be damaged and slight trauma may be caused by the action of some cosmetics that may enhance microbial infection. This may become of particular concern when cosmetics are used around the eyes, on mucous membranes in general, on damaged skin, on children under 3 years, on elderly people and persons showing compromised immune responses. Consequently, two separate categories of cosmetic products are defined in the microbiological quality control limits :

Category 1 : Products specifically intended for children under 3 years, to be used in the eye area and on mucous membranes.

Category 2 : Other products.

Microbial contaminants usually come from two different origins : during production and filling, and during the use of the cosmetic by the consumer. From the moment the cosmetic unit is opened until the last use of the product by the consumer(s), a permanent, variable and additive microbial contamination of the cosmetic is introduced, caused by the domestic environment and contact with the skin of the consumer(s) (hands and body).

Reasons for microbial preservation of cosmetics are :

- to ensure the microbial safety of cosmetics for the consumer,
- to maintain the quality and specifications intended of the product,
- to confirm hygienic and high-quality handling.

Although only a small number of cases of microbiological contamination of cosmetics, leading to microbial infections of the consumer, has been reported, microbial contamination of cosmetic products may spoil them or seriously reduce the intended quality.

In order to ensure the quality of the product and the safety for the consumer, it is necessary to carry out routine microbiological analysis of each batch of the finished product coming on the market. The parameters examined, the criteria and methods used, and the results obtained per batch should be specified in properly filed reports and be taken up in the TIF.

6-4.2 Quantitative and qualitative limits

[based on Colipa 1997, McEwen et al. 2001, US FDA 2001]

It is generally accepted that for cosmetics classified in *Category 1*, the total viable count for aerobic mesophyllic microorganisms should not exceed 10^2 cfu/g or 10^2 cfu/ml in 0.5 g or 0.5 ml of the product.

For cosmetics classified in *Category 2*, the total viable count for aerobic mesophyllic microorganisms should not exceed 10^3 cfu/g or 10^3 cfu/ml in 0.1 g or 0.1 ml of the product.

Pseudomonas aeruginosa, *Staphylococcus aureus* and *Candida albicans* are considered the main potential pathogens in cosmetic products. These specific potential pathogens must not be detectable in 0.5 g or ml of a cosmetic product of *Category 1* and in 0.1 g or 0.1 ml of a cosmetic product of *Category 2*.

It is important to note that the microbial limits mentioned above must be obtained after complete processing of 0.5 g (or 0.5 ml) and 0.1 g (or 0.1 ml) in the case of *Category 1* and *Category 2*, respectively. This is done in order to ensure a statistically significant value of the microbial burden of a cosmetic in the case of positive results. However, smaller amounts of product may be processed in the routinely quality control process if negative results are obtained.

6.4-3 Challenge testing

[based on US Pharmacopoeia 2002, European Pharmacopoeia 2001]

The efficacy of the preservation of a cosmetic product under development has to be assessed experimentally in order to ensure microbial stability and preservation during storage and use. This is done by challenge testing. The latter is mandatory for all cosmetic products that, under normal conditions of storage and use, may deteriorate or form a risk to infect the consumer.

A challenge test consists of an artificial contamination of the finished product, followed by a subsequent evaluation of the decrease in contamination to levels ensuring the microbial limits established for Categories 1 and 2. The microorganisms used in the challenge test may be issued from official collection strains from any state in the EU to ensure reproducibility of the test and are : *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans.*

Nowadays, it is well known that the consistency of challenge tests relies more on the capability of the used microorganisms to contaminate a specific cosmetic product than on the taxonomic status of the microorganisms, their initial concentrations, or the conditions of incubation and media of recovery used. Microorganisms with the capability to contaminate specific cosmetics are the best candidates for use in a challenge test. Consequently, additional "in-house" bacteria and fungi may be used for additional specific purposes of challenge testing. The microcidal activity of preservatives or any other compound in the finished cosmetic must be ruled out in the challenge test by dilution, filtration, the addition of neutralisers or any other means.

The experimental performance of the microbial controls and the challenge tests must be carried out / supervised and validated by a microbiologist.
As mentioned before, the manufacturer must guarantee the efficacy of the preservation of his products experimentally by challenge testing. However, as no legal nor universal challenge test method is available today, it is up to the manufacturer to decide on the details of the test to be used.

6-4.4 Good Manufacturing Practice.

[based on Van Der Maren 1995, Colipa 1994]

In order to comply with Good Manufacturing Practice and Microbial Quality Management, manufacturers of cosmetics have to define and follow specific cleaning, sanitation and control procedures to keep all apparatus and materials appropriately clean and free of pathologic microorganisms. Procedures also include microbiological control of raw materials, bulk and finished products, packaging material, personnel, equipment and preparation and storage rooms.

6-5 REFERENCES

76/768/EEC - Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products.
Official Journal L 262, 27/09/1976 p.169.

93/35/EEC - Council Directive 93/35/EEC of 14 June 1993 amending for the sixth time Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products.
Official Journal L 151, 23/06/1993 p.32.

2003/15/EC - Directive 2003/15/EC of the European Parliament and of the Council of 27 February 2003 amending Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products.
Official Journal L66, 11/03/2003 p.26.

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Brussels, July 1994.

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Technical Guidelines. Microbiological Guidelines.
CTFA Cosmetic, Toiletries and Fragrance Association, Washington DC, November 2001.

SCCNFP/0068/98, Final : Guidelines on the use of human volunteers in compatibility testing of finished cosmetic products, *adopted by the SCCNFP during the plenary session of 23 June 1999*.

SCCNFP/0321/00, Final : Notes of Guidance for Testing of Cosmetic Ingredients for Their Safety Evaluation, 4th revision, *adopted by the SCCNFP during the plenary meeting of 24 October 2000*.

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Doc. EPA/600/P-95/002Fa. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C. (1997).

US FDA (Food and Drug Administration)
Bacteriological Analytical Manual, Chapter 23, August 2001.

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25th Edition, Monograph 1111 (2002).

Van Der Maren L.
Guidelines for good manufacturing practice of cosmetic products (GMPC).
Council of Europe, Health protection of the consumer, ISBN : 92-871-2849-9 (1995).

7. LIST OF OFFICIAL TEXTS OF DIRECTIVE 76/768/EEC INCLUDING ALL TECHNICAL ADAPTATIONS AND AMENDMENTS

Existing EEC Directive	Reference number	Date of signature	EC Publication O.J.		Content (main items)
			Number	Date	
Basic Council Directive	76/768/EEC	27.07.1976	L 262	27.09.1976	Articles 1 to 15 Ann. I: illustrative list by category of cosmetic products. Ann. II: list of forbidden substances. Ann. III: list of restricted substances. Positive List (PL) for cosmetic colouring agents permitted for all uses. Ann. IV: list of restricted substances provisionally allowed. list of cosmetic colouring agents provisionally allowed. Ann. V: list of substances regulated at national level by EC Member States.
1st amendment Council Directive	79/661/EEC	24.07.1979	L 192	31.07.1979	Ann. IV: provisional authorisations prolonged
1st adapting Commission Directive	82/147/EEC	11.02.1982	L 63	06.03.1982	Ann. II: Ban AETT (362)
2nd amendment Council Directive	82/368/EEC	17.05.1982	L 167	15.06.1982	Articles: - provisional authorizations Ann. IV prolonged - new procedure to adapt Annexes (Art. 8.2) - introduction procedure of Prior National Approval limited to 3 years (Art. 8.a) - unavoidable traces of banned materials permitted (Art. 4.2) Ann. III: new version Part 1 Ann. III + IV: new version of PL for cosmetic colouring agents Ann. VI: introduction of PL for preservatives
2nd adapting Commission Directive	83/191/EEC	30.03.1983	L 109	26.04.1983	Ann. II, III, IV, V: - Ba/Sr/Zr lakes - Al/Zr complexes, silver nitrate
3rd adapting Commission Directive	83/341/EEC	29.06.83	L 188	13.07.1983	Ann. II, III, V: hair dyes - ban OPD + salts; 2,4 DAT + salts; permanent listing PPD + salts
4th adapting Commission Directive	83/496/EEC	22.09.1983	L 275	08.10.1983	Ann. VI: addition 36, 45
3rd amendment Council directive	83/574/EEC	26.10.1983	L 332	28.11.1983	Articles: new definition of the date of minimum durability, period reduced to 30 months Introduction Ann. VII: PL for UV filters
5th adapting Commission Directive	84/415/EEC	18.07.1984	L 228	25.08.1984	Ann. II: ban aristolochic acid Ann. III: hydrogen peroxide, hydroquinone, nicomethanol hydrofluoride, silver nitrate

Existing EEC Directive	Reference number	Date of signature	EC Publication O.J.		Content (main items)
			Number	Date	
6th adapting Commission Directive	85/391/EEC	16.07.1985	L 224	22.08.1985	Ann. II: ban specific hydroquinone ethers Ann. III: selenium disulfide; Al/Zr complexes Ann. VI: labelling formaldehyde
7th adapting Commission Directive	86/179/EEC	28.02.1986	L 138	24.05.1986	Ann. II: ban chloroform, TCDD, dimethoxane, sodium pyrithione Ann. III: DMET, 8-hydroxyquinoline Ann. III + IV: new version of PL for cosmetic colouring agents
8th adapting Commission Directive	86/199/EEC	26.03.1986	L 149	03.06.1986	Ann. IV: introduction of other uses 43 Ann. VI: new version of PL for preservatives
9th adapting Commission Directive	87/137/EEC	02.02.1987	L 56	26.02.1987	Ann. II: ban Captan (370), hexachlorophene (371), Minoxidil (372) Ann. III: Methanol, 77288 - 77289 Ann. VI: permanent: 40, deleted: 9, 12, 13
10th adapting Commission Directive	88/233/EEC	02.03.1988	L 105	26.04.1988	Ann. II: ban tribromsalan, retinoic acid, phytolacca, 2,4-DAA, 2,5-DAA, 12140, 26105, 42555 Ann. III: Part 1: etidronic acid, phenoxypropanol Ann. III: Part 2: delete 13065, add Acid Red 195 Ann. IV: Part 2: delete 12700, 44025, 73312 Ann. VI: permanent: 41, 42, 43 deleted: 7, 8, 10, 11, 14, 18, 22, 23, 24
4th amendment Council Directive	88/667/EEC	21.12.1988	L 382	31.12.1988	Articles: - hair dyes excluded from cosmetic colouring agents list - labelling container + packaging - elimination 6 week deadline in Safeguard Clause (Art. 12) Ann. III: becomes only restrictive list Ann. IV: becomes only cosmetic colouring agents PL
11th adapting Commission Directive	89/174/EEC	21.02.1989	L 64	08.03.1989	Ann. II: ban Padimate A, benzoyl peroxide, 2A-4NP, 2A-5NP Ann. III: Part 2: add 8-OH-quinoline Ann. IV: Part 2: delete 15800, 19120, 20470, 21115, 42170, 45190, 47000, 73905, 75660 Ann. V: delete oestrogens Ann. VI: 39 reduce concentration, add 48 (prov.) Ann. VII: new version Part 2
5th amendment Council Directive	89/679/EEC	21.12.1989	L 398	30.12.1989	Articles: CATP procedure prolonged indefinitely
12th adapting Commission Directive	90/121/EEC	20.02.1990	L 71	17.03.1990	Ann. II: ban steroid antiandrogens, zirconium compounds, thyrothricine, acetonitrile, tetrahydrozoline, 13065, 42535, 42640, 61554 Ann. III: lead acetate Ann. IV: delete 21110, 42045, 44045, add Solvent Yellow 98 (prov.) Ann. V: transfers to other Annexes

Existing EEC Directive	Reference number	Date of signature	EC Publication O.J.		Content (main items)
			Number	Date	
13th adapting Commission Directive	91/184/EEC	12.03.1991	L 91	12.04.1991	Ann. II: ban 8-OH-quinoline, pyrithione diS, lidocaine, 12075, 45170 Ann. III: add Mg fluoride Ann. IV: 15585 move to Part 2 Ann. V: transfers to other Annexes Ann. VI: add 27 (prov.) Ann. VII: add 7
14th adapting Commission Directive	92/8/EEC	18.02.1992	L 70	17.03.1992	Prolongation of all provisionally listed substances until 30.06.1992
15th adapting Commission Directive	92/86/EEC	21.10.1992	L 325	11.11.1992	Ann. II: ban 15585, Sr lactate, Sr nitrate, Sr polycarboxylate, Pramocaine, 4-ethoxy-MPD, 2,4-Diamino-phenylethanol, catechol, pyrogallol, nitrosamines, secondary dialkanolamines Ann. III: add Sr chloride, Sr acetate, talc, nitrosamines precursors, H_2O_2 add oral hygiene Ann. III Part 2 + Ann. IV Part 2: nothing listed anymore Ann. VI: 36 sunscreen use with limit, add 51, 29 (prov.) Ann. VII: delete 1, 4, 16
6th amendment Council Directive	93/35/EEC	14.06.1993	L 151	23.06.1993	Articles: - definition modified, - overall safety clause modified, - ban animal testing foreseen, - inventory cosmetic ingredients, - off-pack labelling in some cases, - labelling of product function, - ingredient labelling, - claims concerning animal testing, - requirements to Poison Centres modified, - Product Information required, - notification manufacturing premises, - all Annexes via CATP procedure, - new Annex VIII
16th adapting Commission Directive	93/47/EEC	22.06.1993	L 203	13.08.1993	Ann. II: ban 4 A-2NP Ann. III: warning: gloves for hair dyes + H_2O_2 add (Part 2) Sr peroxide, phenolphthalein Ann. VII: move 33 to prov.
17th adapting Commission Directive	94/32/EC	29.06.1994	L 181	15.07.1994	Ann. II: ban 2-Methyl-MPD Ann. III: talc lab. baby prod. modified, add SrO_2, $Sr(OH)_2$, Ann. VI: add formic acid and its sodium salt, 21 reduction conc. + RO only, delete 26, 27, 28 Ann. VII: add 11 (prov.), delete 24
18th adapting Commission Directive	95/34/EC	10.07.1995	L 167	18.07.1995	Ann. II: ban furocoumarines, ban musk ambrette, ban benzethonium chloride, ban cells, tissues, products of human origin, ban phenolphthalein Ann. VII: Part 1: add octocrylene (10)

Existing EEC Directive	Reference number	Date of signature	EC Publication O.J.		Content (main items)
			Number	Date	
19th adapting Commission Directive	96/41/EC	25.06.1996	L 198	08.08.1996	Ann. II: ban urocanic acid (418) Ann. III: add $Ca(OH)_2$ and LiOH Ann. VI: Part 1: add 52 Ann. VII: Part 1: add 11
20th adapting Commission Directive	97/1/EC	10.01.1997	L 16	18.01.1997	Ann. II: ban bovine, ovine and caprine tissues and fluids from encephalon, spinal cord and eyes, and derivatives
Commission Directive postponing the ban on animal testing	97/18/EC	17.04.1997	L 114	01.05.1997	The ban on animal testing of cosmetic ingredients and their combinations is postponed until 30 June 2000
21st adapting Commission Directive	97/45/EC	14.07.1997	L 196	24.07.1997	Ann. II: ban crude and refined coal tars Ann. VI: Part 1: add benzethonium chloride (53) Ann. VII: Part 1: add octyl methoxy-cinnamate (12)
22nd adapting Commission Directive	98/16/EC	05.03.1998	L 77	14.03.1998	Ann. II: amendment of 419 to derogate tallow derivatives
23rd adapting Commission Directive	98/62/EC	03.09.1998	L 253	15.09.1998	Ann. II: ban of moskene (421) and musk tibetene (422) Ann. VI: Part 1: add benzalkonium chloride (54) Ann. VII: Part 1: add 13, 14, 15, 16, 17, 18, 19, 20
24th adapting Commission Directive	2000/6/EC	29.02.2000	L 56	01.03.2000	Ann. II: adaptation to entry 419 Ann. III: amendment of 1 and 14, add 65 Ann. VI: Part 1: add 55 and 56, Part 2: delete 21 and 29 Ann. VII: Part 1: add 21, 22, 23, 24 and 25, Part 2: delete 5, 17 and 29
25th adapting Commission Directive	2000/11/EC	10.03.2000	L 65	14.03.2000	Ann. II: delete 362, 365, 367, 372, 373, 374, 386, 390, 391, 392, 393 and 394, add 362, 365, 367, 372, 373, 374, 385, 386, 390, 391, 393 and 394
26th adapting Commission Directive	2002/34/EC	15.04.2002	L 102	18.04.2002	Ann. II: amendment of 293, add 423 to 451 Ann. III: Part 1: amendment of 8, 15b, 15c and 16 add 66 Ann. III: add 1 to 62 Ann. VII: Part 1: add 26 and 27
adapting Commission Directive	2003/1/EC	06.01.2003	L 5	10.01.2003	Ann. II: amendment of 419

Existing EEC Directive	Reference number	Date of signature	EC Publication O.J.		Content (main items)
			Number	Date	
Amendment Council Directive	2003/15/EC	27.02.2003	L 66	11.03.2003	Articles: - ban on animal testing with clear deadlines - ban on the use of CMR Cat. 1 and 2 [67/548/EC, Ann.I] ingredients - labelling issues, such as a new requirement for the indication of the date of durability, claims on animal testing, … - inclusion in the product information of any animal testing performed Ann. III: Part 1: add reference nrs° 67 to 92 (fragrance allergens) Ann. VIIa: symbol representing an open cream jar
adapting Commission Directive	2003/16/EC	19.02.2003	L 46	20.02.2003	Ann. III: Part 2: postponement of the date for 61 and 62
adapting Commission Directive	2003/80/EC	05.09.2003	L 224	06.09.2003	Ann. VIIIa: symbol representing an open cream jar
adapting Commission Directive	2003/83/EC	24.09.2003	L 238	25.09.2003	Ann. II: amendment of 178 and 411, delete 382 Ann. III: Part 1: amendment of 14, 60, 61 and 62 add 93,94 and 95 Ann. VI: Part 1: amendment of 36

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2003/83/EC - Commission Directive 2003/83/EC of 24 September 2003 adapting to technical progress Annexes II, III and VI to Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products.
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