Scientific Committee on Consumer Safety

SCCS

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

7TH REVISION

The SCCS adopted this opinion at its 9th plenary meeting
of 14 December 2010.

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 SCCS*" is a document compiled by the members of the Scientific Committee on Consumer Safety (SCCS, replacing the former SCCP, SCCNFP and SCC). The document contains relevant information on the different aspects of testing and safety evaluation of cosmetic ingredients in Europe. It is designed to provide guidance to public authorities and cosmetic industry, in order to improve harmonised compliance with Directive 76/768/EEC[1] and in particular by the 6th (Directive 93/35/EEC[2]) and 7th (Directive 2003/15/EC[3]) 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.
An important new development is the 2009 legislative recast which transforms Directive 76/768/EEC into a Regulation. Although the most relevant provisions thereof are briefly mentioned, it must be emphasised that until 11 July 2013, EC Regulation N°1223/2009 is not yet fully applicable.

The previous revision of the Notes of Guidance took place in 2006 (SCCP/1005/06[4]). 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.

As was also the case in previous revisions, individual SCC(NF)P or SCCS opinions are not provided in detail, but are briefly summarised and clearly referred to.

The "Notes of Guidance" should not be seen as a checklist, but have been compiled to provide assistance in the complex process of the testing and safety evaluation of cosmetic ingredients.
Input of scientists from industry, other scientific committees (SCHER, SCENIHR) and the European Cosmetics Association (Colipa), is gratefully acknowledged.

The Chairperson

1 **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.

2 **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.

3 **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.

4 **SCCP/1005/06**: The SCCP's notes of guidance for the testing of cosmetic ingredients and their safety evaluation, 6th revision, *adopted by the SCCP during the 10th plenary meeting of 19 December 2006*.

SCIENTIFIC COMMITTEE ON CONSUMER SAFETY

ANGERER J.	(DE)	
BERNAUER U.	(DE)	
CHAMBERS C.	(IE)	
CHAUDHRY M.	(UK)	
DEGEN G.	(DE)	
EISENBRAND G.	(DE)*	
GALLI C.	(IT)*	
PLATZEK T.	(DE)	
RASTOGI S.	(DK)	
ROGIERS V.	(BE)	*Vice-Chair*
ROUSSELLE C.	(FR)	
SANNER T.	(NO)	*Vice-Chair*
SAVOLAINEN K.	(FI) *	
VAN BENTHEM J.	(NL)	*Associated scientific advisor*
VAN ENGELEN J.	(NL)	
VINARDELL M.P.	(ES)	
WARING R.	(UK)	
WHITE I.	(UK)	*Chair*

* resigned from the SCCS in 2010

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
BMD	BenchMark Dose
BMDL	BMD Lower limit
BMR	BenchMark Response
BSE	Bovine Spongiform Encephalopathy
BW	Body Weight
CAS n°	Chemical Abstracts Service registry number
Cat.	Category
CI	Colour Index
CLP	Classification, Labelling and Packaging of Substances and Mixtures
CMR	Carcinogenic, Mutagenic, toxic to Reproduction
Colipa	European Cosmetics Association (formerly the 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 mixture 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 mixture, 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 mixture 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]
CPDB	Carcinogen Potency Database
CTA	Cell Transformation Assay
DA_a[1]	Dermal Absorption reported as amount/cm²
DA_p[1]	Dermal Absorption expressed as a percentage
Dermal / percutaneous absorption	The **percutaneous/dermal absorption** process is a global term which describes the passage of compounds across the skin. This process can be divided into three steps: - **penetration** is the entry of a substance into a particular layer or structure such as the entrance of a compound into the stratum corneum; - **permeation** is the penetration through one layer into another, which is both functionally and structurally different from the first layer; - **resorption** is the uptake of a substance into the vascular system (lymph and/or blood vessel), which acts as the central compartment [WHO 2005]
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 [EC B.26]
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 EC B.26]
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 Community
EC Number	EC number, meaning either EINECS number, ELINCS number, NLP number or EC Number appointed under REACH procedure

[1] Used in the calculation of the Systemic Exposure Dosage (see section 3-7.3).

ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EChA	European Chemicals Agency
ECVAM	European Centre for the Validation of Alternative Methods
ED	Endocrine Disruptor
EEC	European Economic Community
EFSA	European Food Safety Authority
EINECS	European INventory of Existing commercial Chemical Substances
ELINCS	European LIst of Notified Chemical Substances
ESAC	ECVAM Scientific Advisory Committee
EST	Embryonic Stem cell Test
EST-1000	Epidermal Skin Test-1000
EU	European Union
F^1	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]
GHS	Globally Harmonised System of classification and labelling of chemicals
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
HPV	High Production Volume
HT_{25}	Human dose-descriptor, derived from T_{25} and based on comparative metabolic rates [Sanner et al. 2001]
ICCG	Inter-Committee Coordination Group
ICE	Isolated Chicken Eye
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
IRE	Isolated Rabbit Eye
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) [EC B.1bis]
LED	Lowest Effective Dose

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]
MLA	Mouse Lymphoma Assay
MM	MicroMass
MN	MicroNucleus
MoE	Margin of Exposure
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
NLP	No Longer Polymer
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 [based on EC B.26]
NRU	Neutral Red Uptake
OECD	Organisation for Economic Co-operation and Development
PCPC	Personal Care Products Council (formerly CTFA - Cosmetic, Toiletry and Fragrance Association)
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
REACH	Registration, Evaluation, Authorisation and restriction of CHemicals
RHE	Reconstructed Human Epidermis
RIVM	RijksInstituut voor Volksgezondheid en Milieu
rLLNA	reduced Local Lymph Node Assay
S9	Co-factor supplemented post-mitochondrial fraction, prepared from the livers of rodents treated with enzyme-inducing agents [EC B.10]
SC	Stratum Corneum
SCC	Scientific Committee on Cosmetology
SCCNFP	Scientific Committee on Cosmetic products and Non-Food Products intended for consumers
SCCP	Scientific Committee on Consumer Products
SCCS	Scientific Committee on Consumer Safety
SCE	Sister Chromatid Exchange
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCHER	Scientific Committee on Health and Environmental Risks
SD	Standard Deviation of the mean
SED	Systemic Exposure Dosage
SHE	Syrian Hamster Embryo
SI	Stimulation Index

SIT	Skin Irritation Test
SRM	Specified Risk Material
SSA[1]	Skin Surface Area
SSC	Scientific Steering Committee
Syndet	Synthetic detergent
T_{25}	Animal dose-descriptor; chronic dosage rate that will give 25% of the animal's tumours at a specific tissue site after correction for spontaneous incidence [Dybing et al. 1997]
TD_{50}	The chronic dosage rate (in mg/kg bw per day) which would halve the actuarially adjusted percentage of tumour-free animals at the end of a standard experiment time -the "standard lifespan"- for the species. [Peto et al. 1984]
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) [EC B.41]
Valid method	A technique that has not necessarily gone through the complete validation process, but for which sufficient scientific data exist demonstrating its relevance and reliability. [based on Rogiers 2003]
Validated method	A method for which the relevance and reliability are established for a particular purpose (in most cases 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] These methods are taken up in Regulation (EC) No 440/2008 and/or published as OECD Technical Guidelines*.
VIS	VISible light (wavelength 400-800 nm)
WEC	Whole Embryo Culture
WHO	World Health Organisation

* available through http://www.oecd.org/document/55/0,2340,en_2649_34377_2349687_1_1_1_1,00.html, consulted December 2010.

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

TABLE OF CONTENTS

1. INTRODUCTION 11

2. THE SCIENTIFIC COMMITTEE ON CONSUMER SAFETY 13

2-1 Historical background 13

2-2 Mandate 14

2-3 Rules of Procedure 14

2-4 Outcome of discussions 15

2-4.1 The "Notes of Guidance" 15
2-4.2 The status of cosmetic ingredients included in Annexes II, III, IV, VI and VII of Directive 76/768/EEC 16
2-4.3 General issues taken up in the "Notes of Guidance" 16

3. SAFETY EVALUATION OF COSMETIC INGREDIENTS 19

3-1 Introduction 19

3-2 Safety evaluation procedure of cosmetic ingredients as applied by the SCCS 22

3-3 Chemical and physical specifications of cosmetic ingredients 23

3-3.1 Chemical identity 24
3-3.2 Physical form 24
3-3.3 Molecular weight 25
3-3.4 Characterisation and purity of the chemical 25
3-3.5 Characterisation of the impurities or accompanying contaminants 25
3-3.6 Solubility 25
3-3.7 Partition coefficient (Log P_{ow}) 25
3-3.8 Additional relevant physical and chemical specifications 25
3-3.9 Homogeneity and stability 26
3-3.10 Functions and uses 26

3-4 Relevant toxicity studies on cosmetic ingredients 26

3-4.1 Acute toxicity 27
3-4.2 Corrosivity and irritation 28
3-4.3 Skin sensitisation 31
3-4.4 Dermal / percutaneous absorption 32
3-4.5 Repeated dose toxicity 34
3-4.6 Mutagenicity/genotoxicity 35
3-4.7 Carcinogenicity 37
3-4.8 Reproductive toxicity 38
3-4.9 Toxicokinetic studies 38
3-4.10 Photo-induced toxicity 39
3-4.11 Human data 40

3-5 Toxicological requirements for inclusion of a substance in one of the Annexes to Directive 76/768/EEC (evaluated by the SCCS) 41

3-5.1 General toxicological requirements 41
3-5.2 Annex II 42
3-5.3 Annex III 42
3-5.4 Annex IV 42
3-5.5 Annex VI 43
3-5.6 Annex VII 43
3-5.7 Requirements for partial evaluations 43

3-6 Basic requirements for cosmetic ingredients (which are evaluated by individual safety assessors) 43

3-6.1 General toxicological requirements 43
3-6.2 Identification of mineral, animal, botanical and biotechnological ingredients 44
3-6.3 Fragrance materials 46
3-6.4 Potential endocrine disruptors 46
3-6.5 Animal-derived ingredients, incl. BSE-issues 48
3-6.6 CMR-ingredients 49
3-6.7 Nanomaterials 51

3-7 General principles for the calculation of the Margin of Safety and lifetime cancer risk for a cosmetic ingredient 52

3-7.1 Introduction: definitions 52
3-7.2 Calculation of the Margin of Safety of a cosmetic ingredient 53
3-7.3 Dermal absorption issues in the calculation of the SED 56
3-7.4 MoS for children 57
3-7.5 The Threshold of Toxicological Concern (TTC) 58
3-7.6 Assessment of carcinogens 58

3-8 The specific assessment of hair dyes and hair dye components 62

3-8.1 Hazard and risk assessment of hair dyes in general 62
3-8.2 Step-wise regulation of hair dyes 62
3-8.3 MoS calculations for hair dye formulations 63
3-8.4 Mutagenicity / genotoxicity testing of hair dye substances and reaction products 63

4. SAFETY EVALUATION OF FINISHED COSMETIC PRODUCTS 65

4-1 Introduction 65

4-2 Categories of cosmetic products and exposure levels in use 65

4-3 Guidelines for the safety evaluation of finished cosmetic products 72

4-3.1 Introduction 72
4-3.2 Toxicological profile of the ingredients 72
4-3.3 Stability and physical and chemical characteristics of the finished cosmetic product 72
4-3.4 Evaluation of the safety of the finished product 73

4-4 Guidelines on microbiological quality of the finished cosmetic product .. 74

4-4.1 Preamble 74
4-4.2 Quantitative and qualitative limits 74
4-4.3 Challenge testing 75
4-4.4 Good Manufacturing Practice 75

5. REFERENCE LIST 76

APPENDIX 1: LISTS OF INGREDIENTS 100

1. Introduction 100

2. Annexes II, III, IV, VI and VII to the Cosmetic Products Directive 100

3. INVENTORY OF INGREDIENTS USED IN COSMETIC PRODUCTS 102

4. CosIng - EC information on cosmetic ingredients 103

5. Part 3 of Annex VI to Regulation (EC) No 1272/2008 103

6. References 104

APPENDIX 2: STANDARD FORMAT OF THE OPINIONS 105

1. INTRODUCTION

According to Article 1 of Council Directive 76/768/EEC and its amendments, *a **cosmetic product** shall mean any substance or mixture 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[1]].

In June 1982, long before 93/35/EEC was implemented as the 6th Amendment to Directive 76/768/EEC, a pioneer document was issued by the former SCC dealing with "Guidelines for the toxicity testing of cosmetic ingredients" (Report EUR 8794). 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 SCCS using data obtained from animal studies (*in vivo*), *in vitro* experiments, QSAR (quantitative structure activity relationship) calculations, clinical studies, epidemiological studies and accidents. The physical and chemical data of the compounds under investigation are also taken into consideration.

With the implementation of Directive 2003/15/EC[2], the need for validated *in vitro* tests for the safety evaluation of cosmetic ingredients and products became crucial.

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], is incorporated.
In particular, the SCCS gives special attention to those alternative methods that are suitable for the safety testing of cosmetic ingredients. These are taken up in the appropriate chapters.

---

1 **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.

2 **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 SCCS would like to stress that currently available *in vitro* methods only constitute a fraction of the alternative methodology meant by Russell et al [1959[1]], 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.

The revised "Notes of Guidance" are mainly concerned with testing and safety evaluation of the cosmetic ingredients listed in Annexes III, IV, VI, and VII of Directive 76/768/EEC and those for which safety concerns have been expressed. However, they 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 PIF (product information file) for a finished cosmetic product as required by Directive 93/35/EEC and EC Regulation N°1223/2009.

The "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.

As the science of toxicology advances, as validated alternative methods become adopted and as legislative changes are introduced, the "Notes of Guidance" will be revised as scientifically required.

[1] 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 CONSUMER SAFETY

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**), was established by Commission Decision 97/579/EC. It was composed of independent scientists from different fields of competence, collectively covering the widest possible range of expertise. Between 1997 and 2004, the SCCNFP adopted a series of scientific opinions related to the improvement of the safety evaluation of cosmetic ingredients.
[ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04/index_en.htm]

In 2004, the SCCNFP was replaced by the Scientific Committee on Consumer Products (**SCCP**) through Commission Decision 2004/210/EC. This replacement formed part of a larger-scale reorganisation of the EU Scientific Committees in the field of consumer safety, public health and the environment, during which the existing 8 Committees were disbanded and reorganised.

Three scientific committees were established:

i. Scientific Committee on Consumer Products (SCCP)
ii. Scientific Committee on Health and Environmental Risks (SCHER)
iii. Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR)

The coordination between the SCCP, the SCHER and the SCENIHR was proposed to be done by the Inter-Committee Coordination Group (**ICCG**).

Between 2004 and 2008, the SCCP continued the work previously performed by the SCC and SCCNFP.
[http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccp_opinions_en.htm#1]

Finally, in 2008, the three above-mentioned scientific committees were renewed[1] and the SCCP's name was changed into SCCS (Scientific Committee on Consumer Safety). In addition to the SCCS, SCENIHR and SCHER, a Pool of scientific advisors on risk assessment was also established, with the specific task to assist the members of the scientific committees in their work. In 2009, the names of the appointed members of the three committees and the Pool were published in the Official Journal of the European Union[2].

[1] **Commission Decision 2008/721/EC** of 5 September 2008 setting up an advisory structure of Scientific Committees and experts in the field of consumer safety, public health and the environment and repealing Decision 2004/210/EC.
Official Journal L 241, 10/09/2008 p.21.

[2] **Commission Decision 2009/146/EC** of 19 February 2009 on the appointment of the members and advisors of the Scientific Committees and the Pool set up by Decision 2008/721/EC.
Official Journal L 49, 20/02/2009 p.33.

2-2 MANDATE

The SCCS's field of competence is defined in Commission Decision 2008/721/EC, which states that the committee 'shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.)'.

In addition, the Commission may request from the Committee:
- advice on any matter of particular relevance to consumer safety and public health not falling within the mandate of other Community bodies;
- rapid advice on the state of scientific knowledge concerning specific risks in case of urgent needs;
- the identification of research needs and the assessment of research results in relation to the subject areas covered by its fields of competence;
- to be part of thematic networks with other Community bodies or scientific organisations, in order to monitor and contribute to the development of scientific knowledge on risks in the fields of competence.

Also, upon its own initiative, the Committee shall draw the Commission's attention to a specific or emerging problem falling within its remit, which is considered to potentially pose an actual or potential risk to consumer safety, public health or the environment.
Finally, in agreement with the Commission, the SCCS may decide to set up thematic workshops, organised by the Committee's secretariats, in order to review data and scientific knowledge on particular risks or on broad risk assessment issues. At the request of the Commission, they shall produce reports, position papers or conclusions resulting from these workshops.

The work of the SCCS can be divided in two main domains, namely matters related to cosmetic ingredients and products and those related to other non-food consumer products.
Whenever cosmetic ingredients are concerned, the consultation of the SCCS is **compulsory**[1], whereas it is **not compulsory** in the domain of other non-food products.

2-3 RULES OF PROCEDURE

The Rules of Procedure of the SCCS, SCHER and SCENIHR were jointly adopted by the Scientific Committees on 18 December 2009[2].

In order to efficiently fulfil its extensive mandate, the SCCS regularly sets up working groups on particular subjects of interest. These subgroups operate independently under an appointed chairperson (SCCS-member) and consist of SCCS members complemented with experts from the official Pool of scientific advisors and/or external experts in the requested field of competence. Working groups for example deal with: Cosmetic Ingredients (individual ingredient evaluations, with the exception of hair dyes); Hair Dyes; Methodologies (alternative methods and Notes of Guidance); Nanomaterials; Sensitisation and Fragrances; and other topics according to the needs.

Once the experts of the Working Groups have adopted a final version of their scientific report(s), they present it to the SCCS plenary meeting which adopts the texts to be officially published. Many opinions are placed on the Commission's website[3] for comments before ultimate finalisation. This allows stakeholders to post comments which are subsequently considered by the SCCS and incorporated when considered appropriate.

1 See Article 8.2 of Directive 76/768/EEC and its amendments.
2 http://ec.europa.eu/health/scientific_committees/docs/rules_procedure_en.pdf
3 http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm, consulted December 2010

This method of working with subgroups not only relieves the workload from the members of the SCCS, but equally and importantly allows discussing the individual topics with the suitable experts in the field of interest, thus increasing the scientific quality of the opinions issued.

2-4 OUTCOME OF DISCUSSIONS

Before 1997, the opinions adopted by the Scientific Committee on Cosmetology at the Commission's request were included in EC-Reports (EUR 7297, 8634, 8794, 10305, 11080, 11139, 11303, 14208). Between 1997 and 2004, all SCCNFP opinions have been published on the Internet and can be accessed through the Committee's Website[1]. All SCCP / SCCS opinions can easily be located through the ingredient's substance category involved and the adoption date.

It must be emphasised that the SCC(NF)P / SCCS opinions and statements not only refer to cosmetic ingredients included in Annexes II, III, IV, VI and VII of Council Directive 76/768/EEC, but also to 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 responsibilities of the former SCC(NF)P and the present SCCS is 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 successors SCCNFP, SCCP and now SCCS, have adopted, in this respect, the following opinions:

- Notes of Guidance for the toxicity testing of cosmetic ingredients:
  28 June 1982, EU Report 8794.
- Notes of Guidance for testing of cosmetic ingredients for their safety evaluation:
  1st Revision: SPC/803/5/90,
  2nd Revision: DGXXIV/1878/97,
  3rd Revision: SCCNFP/0119/99,
  4th Revision: SCCNFP/0321/00.
- Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation:
  5th Revision: SCCNFP/0690/03,
  6th Revision: SCCP/1005/06.

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 Commission Regulation No 440/2008 (former Annex V to the Directive 67/548/EEC). They enclose the basic toxicity testing procedures needed to evaluate different human health-related toxicological endpoints and are internationally accepted as being the result of long-term scientific agreement. The procedures to be followed for chemical substances include particularly *in vivo* animal models and a limited number of *in vitro* models.
Furthermore, the SCCS commonly accepted and still accepts testing procedures in accordance with the OECD (Organisation for Economic Co-operation and Development) Guidelines, and, on a case-by-case basis, well documented scientifically justified methods based on *in vitro* models or other 3R-alternative procedures.

The SCCS, as its predecessors, strives to work pro-actively. An example is the early acceptance by the SCCNFP of the *in vitro* study on dermal / percutaneous absorption using

human/pig skin. Guidelines were established early onwards [SCCNFP/0167/99] and reviewed on several occasions [SCCNFP/0750/03, SCCP/0970/06, SCCS/1358/10].

Over the years, several 3R-alternative methods have been developed and validated. These are commonly taken up in Commission Regulation No 440/2008. The latter not only includes Reduction and Refinement measures, but also Replacement methods. In view of the fact that in the cosmetic field the 7th Amendment [2003/15/EC] to the Cosmetics Directive [76/768/EEC] 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 3R-validated alternatives and in particular to replacement methods in the safety evaluation of cosmetic ingredients and finished products.

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

Between its establishment in 1997 and its disbandment in 2004, the SCCNFP provided opinions on more than 400 chemical substances and/or their mixtures and the SCCP has added more than 150 opinions to that list. The majority of these opinions have been adopted into Cosmetic Legislation as modifications of the Annexes to Directive 76/768/EEC (Art. 8.2 and Art. 10 of Directive 76/768/EEC).

The actual status of all annexes is shown below:

	STATUS DECEMBER 2010
Annex II (forbidden substances)	1365 entries
Annex III, Part 1 (restrictions)	208 entries
Annex III, Part 2 (restrictions, provisionally allowed)	31 entries
Annex IV, Part 1 (list of colouring agents)	153 colourants
Annex IV, Part 2 (colouring agents, provisionally allowed)	empty
Annex VI, Part 1 (preservatives)	54 preservatives
Annex VI, Part 2 (preservatives, provisionally allowed)	empty
Annex VII, Part 1 (UV filters)	26 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 Directive 76/768/EEC, some specific general issues have been addressed by the former SCC(NF)P and the actual SCCS. Examples of these include:

Guidelines for human testing in cosmetic science		
- potentially skin irritating cosmetic ingredients or mixtures		SCCNFP/0003/98
- compatibility testing of finished cosmetic products (potentially skin irritating)		SCCNFP/0068/98 SCCNFP/0245/99
- predictive testing of potential skin sensitisers		SCCNFP/0120/99
- classification of skin sensitisers and grading of test reactions		SCCP/0919/05
Alternative methods in the safety assessment of cosmetics		
- general opinion on the use of alternatives for cosmetic ingredients and mixtures		SCCNFP/0103/99
- status report on available alternative methods	2002	SCCNFP/0546/02
	2007	SCCP/1111/07
	2010	SCCS/1294/10
- comments on ECVAM report for establishing the timetable for phasing out animal testing		SCCNFP/0834/04
- comments on the *in vitro* EpiSkin™ assay (skin irritation)		SCCP/1145/07
- genotoxicity/mutagenicity testing without animals		SCCP/1212/09
Cosmetic ingredients of animal / human origin		
- Bovine Spongiform Encephalopathology (BSE) issues i.e. Annex II n° 419 of to Directive 76/768/EEC	2001	SCCNFP/0451/01 SCCNFP/0521/01
	2002	SCCNFP/0552/02 SCCNFP/0612/02
	2003	SCCNFP/0724/03
- amino acids obtained by hydrolysis of human hair		SCCP/0894/05
- animal by-products not intended for human consumption		SCCP/0933/05
CMR (Carcinogenic, Mutagenic, toxic to Reproduction) issues		
- CMR substances in cosmetic products	2001	SCCNFP/0474/01
	2004	SCCNFP/0825/04
	2005	SCCP/0888/05 SCCP/0913/05
- new CMR classification according to Regulation 790/2009		SCCS/1284/09
Safety assessment of hair dyes and colourants		
- foreseeable use of hair dyes		SCCNFP/0059/98
- use of hair dyes and (bladder) cancer risk	2001	SCCNFP/0484/01
	2004	SCCNFP/0797/04
	2005	SCCP/0930/05
- safety review of the use of certain azo-dyes		SCCNFP/0495/01
- assessment strategies for hair dyes	2002	SCCNFP/0553/02
	2005	SCCP/0959/05
- genotoxicity/mutagenicity testing of hair dyes	2002	SCCNFP/0566/02
	2003	SCCNFP/0720/03
	2006	SCCP/0971/06
- re-evaluation of hair dyes listed in Annex III		SCCNFP/0635/03
- hair dyes without file submitted		SCCNFP/0807/04
- reaction products from hair colouring ingredients	2004	SCCNFP/0808/04
	2005	SCCP/0941/05
	2006	SCCP/1004/06
	2010	SCCS/1311/10
- hair dyes and skin sensitisation	2006	SCCP 2006
	2007	SCCP/1104/07

The inventory of cosmetic ingredients (INCI-list)		
- status report		SCCNFP/0098/99
- *pseudo* INCI names of botanicals		SCCNFP/0099/99
- update of the inventory of ingredients		SCCNFP/0299/00
		SCCNFP/0389/00
Safety of infants and children		
- calculation of the Margin of Safety for children		SCCNFP/0557/02
- fluorine compounds in oral hygiene products	2005	SCCP/0882/05
	2009	SCCP/1214/09
Fragrance allergy in consumers		
- fragrance allergy in consumers	1998	SCCNFP/0017/98
	2001	SCCNFP/0450/01
- prohibited/restricted perfumery materials	2000	SCCNFP/0320/00
		SCCNFP/0392/00
	2003	SCCNFP/0770/03
		SCCNFP/0771/03
	2006	SCCNFP/1023/06
- sensitisation quantitative risk assessment (QRA)		SCCP/1153/08
Risk and health effects: miscellaneous		
- hypoallergenic claims on cosmetic products		XXIV/1895/98
- potentially estrogenic effects of UV filters		SCCNFP/0483/01
- tattoos, body piercing and related practices		SCCNFP/0753/03
- sunbeds for cosmetic purposes (UV-radiation)		SCCP/0949/05
- tooth whitening products		SCCP/0974/06
- nanomaterials in cosmetic products		SCCP/1147/07
- genotoxic and carcinogenic substances		SCHER/SCCP/ SCENIHR 2009
- Threshold of Toxicological Concern (TTC)		SCCP/1171/08

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 Directive 2003/15/EC, the 7th amendment to the Cosmetic Products Directive [76/768/EEC].
After 7 amendments and more than 50 adaptations to technical progress over the years, the Cosmetic Products Directive has recently been recast into a 'Regulation on Cosmetic Products' [2009/1223/EC]. Although the majority of the provisions stated in this new Regulation (EC) only apply from 11 July 2013 onwards, these Notes of Guidance will already mention some important points to inform the reader on the upcoming changes. As the recast takes the form of a Regulation, its provisions will be of application in all Member States, without being subject to potential alterations when translated into national legislations.

The legal basis for the safety evaluation of cosmetic products can be found in Articles 2, 4.a.1 and 7a (d) of Dir. 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 authorised 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 methods listed in Commission Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals

(REACH) *(OJ L142, 31.5.2008, p.1)* 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 (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, germ cell mutagenic or toxic for reproduction, of category 1A, 1B and 2, under part 3 of Annex VI to Regulation (EC) No 1272/2008 ... (OJ L353, 31.12.2008, p.1) shall be prohibited. ... A substance classified in category 2 may be used in cosmetics if the substance has been evaluated by the Scientific Committee on Consumer Safety (SCCS) 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 6th 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 Dir. 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 6th 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 7th 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.
The recast of the Cosmetic Products Directive does not foresee any changes with regard to the imposed deadlines for animal testing; neither does the document introduce significant changes with regard to the safety assessment requirements. The new Annex I is added for clarification, but the major principles remain in place [2009/1223/EC].

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

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

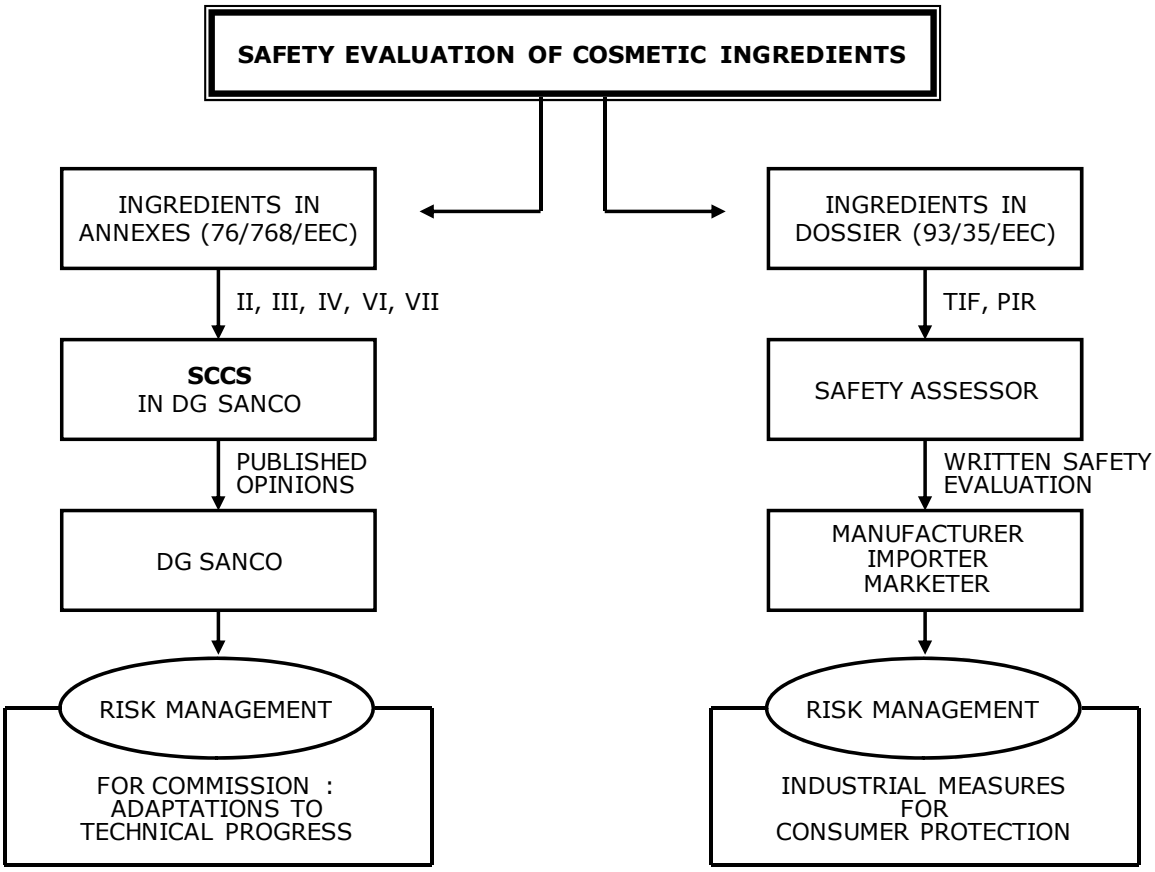

Fig.1: Existing two ways in the safety evaluation of cosmetic ingredients in the EU.

It is primarily the substances in Annexes II, III, IV, VI and VII that fall under the responsibility of the SCCS. 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 SCCS 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 lowest dosage at which an adverse effect is observed (LOAEL) is used. In the case of non-threshold carcinogens, a dose-descriptor (e.g. T_{25}) is determined [Dybing et al. 1997].

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 substance under investigation causes damage to human health and the level of risk, 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 usually 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 assessment of carcinogens is described in Section 3-7.6.

Risk characterisation is followed by **risk management** and **risk communication**, which are not the tasks of the SCCS, but of the European 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 [Barile 2008, Beck et al. 2008, Rogiers 2010]. 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 SCCS 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 to be evaluated by the SCCS should be:

1) chemical identity;
2) physical form;
3) molecular weight;
4) characterisation and 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;
9) homogeneity and stability;
10) function and uses.

The information from these points 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 SCCS [SCCNFP/0633/02].
Preference is clearly given to measured parameters of a couple of relevant batches compared to calculated values (e.g. log P_{ow}) or literature data, since often other batches were tested, with different impurity profiles.

In the following chapter, the methods are (where relevant) accompanied by their corresponding reference number in Regulation (EC) No 440/2008[1] [2008/440/EC].

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 EC number (see Appendix 1 for more detail) should be given.

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 processes and/or purification steps used (see also section 3-6.2).

In the case of a mixture 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/or 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, …

[1] Officially replaces Annex V to Dir. 67/548/EEC.

3-3.3 Molecular weight

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

3-3.4 Characterisation and purity of the chemical

The experimental conditions of the techniques used for the chemical characterisation (UV, IR, NMR, MS, elemental analysis, etc) as well as the resulting spectrum, chromatogram etc. should be provided.
The degree of purity must be clearly defined. The validity of the analytical methodology used, must be shown.
The substance(s) 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 concentrations.

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 neither other impurities nor an increase in the impurities (chemically defined or technically unavoidable, potentially affecting the safety of the finished products) are present in the representative commercial material.

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, specifying pH and temperature.
In case of a calculated value, the method should be specified.
The P_{ow} strongly depends on the pH, especially for ionisable molecules, zwitterions etc. Therefore, a single calculated value of Log P_{ow}, usually without any reference to the respective pH, cannot be correlated to physiological conditions and to the pH conditions of the dermal absorption studies.

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 solvents, including receptor fluids (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], relative 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 temperature [EC A.1], pK_a (..% in ..., at ..°C), ...
- for gases: density [EC A.3] (at ..°C), auto-ignition temperature [EC A.15], …

- in case of a UV light absorbing ingredient, the UV light absorption spectrum of the compound should always be included. It is self-evident that for UV absorbers and UV-filters, this spectrum is absolutely indispensable.

3-3.9 Homogeneity and stability

Homogeneity of the test solutions with respect to the content of the test substance, under experimental conditions, should be provided.
The stability of the test substance under the experimental conditions of various studies should be reported. In addition, the stability of the test substance under storage conditions as well as in typical cosmetic formulations should also be provided.

3-3.10 Functions and uses

For cosmetic ingredients under study, concentration, function and mode of action in marketed cosmetic products should be reported.
In addition, other uses and the concentrations involved should be described (e.g. consumer products, industrial products).

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 still 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 or estimation of "LD_{50}-values". These are mainly used for classification and labelling purposes, as described in Annex VI to Directive 67/548/EEC [2001/59/EC]. The classification and labelling system was changed and largely adapted to the United Nations Globally Harmonised System of Classification and Labelling of Chemicals (the UN GHS)[1]. Therefore a new Regulation on the Classification, Labelling and Packaging of Substances and Mixtures (CLP) was issued in 2008 [2008/1272/EC]. This new GHS system changes some thresholds for classification of substances and mixtures and has consequences for the CMR terminology (see 3-6.6), but it still involves the use of experimental animals.

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 3R-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).

[1] http://www.unece.org/trans/danger/publi/ghs/ghs_welcome_e.html, consulted December 2010

In this respect, some 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, skin irritation, mutagenicity, photomutagenicity, phototoxicity, and dermal absorption. However, due to a variety of reasons, including the complexity of the vertebrate organism, there are presently neither validated *in vitro* replacement methods for the repeated dose animal toxicity studies (including reproductive and developmental toxicity), nor relevant proposals ready for prevalidation/validation, available [Worth et al. 2002, Rogiers 2002, Rogiers and Pauwels 2005, JRC 2010].
Through the provisions of its 7th Amendment [2003/15/EC], the European cosmetic legislation prohibits the marketing of finished products containing an ingredient that has been subject to any animal testing after 2013 in order to meet the requirements of Dir. 76/768/EEC, its amendments and adaptations to technical progress. Therefore the SCCS and its predecessors have closely followed up the progress made with regard to the development and validation of alternative methods. With the aim of providing an objective overview of the status of alternative methods/strategies and the prospects, the committee issues on a regular basis a memorandum on this particular subject [SCCNFP/0103/99, SCCNFP/0546/02, SCCP/1111/07, SCCS/1294/10]. In addition to validated alternative methods, the SCCS may accept, on a case-by-case basis, , also "valid" methods for the safety assessment of cosmetic ingredients. Such valid methods have not necessarily gone through the complete validation process, but the Committee may consider them acceptable when they have a sufficient amount of experimental data proving their relevance and reliability.
According to the 6th 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 Regulation (EC) No 440/2008 and by its OECD** (Organisation for Economic Co-operation and Development) **number**.
For every **animal study** provided, it is essential that **the date of the experiment** is stated. This date not only may explain certain shortcomings in the introduced studies, but can also be used to follow-up the performance of reduction, refinement and replacement alternative methods once they have been officially accepted.

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].

1) Acute oral toxicity

The *in vivo* acute oral toxicity test was originally developed to determine the LD_{50}-value of the compound under investigation. As well in the current as in the future dangerous substances legislation, this LD_{50}-value triggers the classification of the compound [2001/59/EC, 2008/1272/EC].

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**:

- The **fixed dose method** [EC B.1 bis, OECD 420] abandons lethality as an 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.
- 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.

- 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.

2) Acute inhalation toxicity

For acute inhalation toxicity, OECD guideline 436 describes the **acute toxic class** method by the inhalation route [OECD 436].

3) Acute dermal toxicity

No alternatives for the *in vivo* acute dermal toxicity assay [EC B.3, OECD 402] are available.

Usually acute toxicity data of cosmetic ingredients are already available as a result of compliance with the provisions of the 7th amendment to Directive 67/548/EEC on the notification, classification and labelling of dangerous substances [92/32/EEC] and/or through REACH requirements [2006/1907/EC]. Nevertheless, cosmetic products containing ingredients that have been subject to acute toxicity testing after 11 March 2009 to meet the requirements of the Cosmetic Products Directive, are not allowed on the EU market.

3-4.2 Corrosivity and irritation

1) Skin corrosivity and skin irritation

Skin corrosion or dermal 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 [EC B.4, 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 pH, the presence of "neutralising" substances, the excipient used, the exposure route, the conditions of use, etc.

For skin corrosion testing, at present 5 validated *in vitro* alternatives are taken up in Regulation (EC) No 440/2008 [2008/440/EC]:

1) **TER test** (rat skin transcutaneous electrical resistance test) [EC B.40, OECD 430]
2) **EpiSkin™** [EC B.40bis, OECD 431]
3) **EpiDerm™** [EC B.40bis, OECD 431]
4) **SkinEthic™** [EC B.40bis, OECD 431]
5) **EST-1000** (epidermal skin test-1000) [EC B.40bis, 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. It is described in OECD Draft Guideline 435 [OECD 435], which provides a generic description of the components and procedures of an artificial membrane barrier test method for corrosivity assessment. Although the Corrositex™ test 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 was considered to be only useful for acids and bases [ESAC 2000].

Skin irritation or dermal irritation is defined as reversible damage of the skin following the application of a test substance for up to 4 hours. Originally, the standard skin irritation test consisted of an *in vivo* test method involving the use of three to six rabbits. Over the years, the test method has been subject to refinement and reduction measures, bringing the number of animals down from maximum six to maximum three, and now involving a number of steps to be taken before the *in vivo* study can even be envisaged [EC B.5, OECD 405]. These steps consist of:

- the evaluation of existing human and animal data;
- the analysis of structure activity relationships;
- a study of physicochemical properties and chemical reactivity (e.g. substances with a pH $\leq$ 2.0 or $\geq$ 11.5 will be considered as corrosive without *in vivo* testing);
- looking at available dermal toxicity data;
- taking into account the results from *in vitro* and *ex vivo* tests [EC B.4, OECD 404].

Recently a number of *in vitro* skin irritation tests have been officially validated:

1) **EpiSkin™**
2) **Modified Epiderm™ Skin Irritation Test** (SIT)
3) **SkinEthic™ Reconstructed Human Epidermis** (RHE)

A new OECD guideline on *in vitro* skin irritation making use of the reconstructed human epidermis (RHE) test method, is in draft version [OECD 2009] and the recently published EC. B46 counterpart clearly mentions that the test results, depending on information requirements, may allow determining the skin irritancy of substances as a stand-alone replacement test within a testing strategy, in a weight of evidence approach [EC B.41].
Taking the Episkin™ method as an example, however, the SCCS expressed concerns with regard to potential interference with the colour formation by reducing substances, hair dyes and colorants [SCCP/1145/07].
Industry responded by providing additional data on:

1) the EpiSkin™ method applied on a set of 15 UV-filters/preservatives/skin conditioning agents,
2) a modified Episkin™ assay (including an additional negative control) on a set of 22 hair dye substances and 4 color ingredients.

Although the results for the first set of 15 compounds revealed a relatively high correlation between *in vivo* and *in vitro* data, only 2 of the 3 irritating substances *in vivo*, could be identified as irritants by the Episkin™ method.

Of the 22 hair dye substances tested, only 2 were *in vivo* irritants and the adapted Episkin™ assay failed to identify them as such. The 4 color ingredients were *in vivo* irritants but two of them interfered with the test system at the *in vivo* irritating concentration, meaning that a dilution needed to be tested instead. The SCCS expressed the opinion that the modified Episkin™ method did not provide sufficient proof that the MTT test could be used as a suitable endpoint to test color ingredients/hair dye substances for their potential skin irritative properties. A different endpoint, not involving optical density quantification, should be envisaged [SCCS/1392/10].

In the light of the imposed testing ban on cosmetic ingredients, Colipa proposed a decision tree approach for the integration of alternative approaches into tiered testing strategies for hazard and safety assessment of cosmetic ingredients and their use in products. Two separate decision trees are put forward [Macfarlane et al. 2009]:

- A decision tree for hazard identification of the neat test substance, where physicochemical properties, read-across data, QSAR results and *in vitro* skin corrosion data may lead to a classification as corrosive. If all these assays fail to indicate the substance as a skin corrosive, a validated *in vitro* skin irritation assay may either trigger a classification of irritant or non-irritant.
- A decision tree for risk assessment of the neat ingredient in the final formulation(s), where the measured formulation's skin irritancy in an *in vitro* skin irritation test is to be

compared against the measured irritancy of a benchmark control. The last step in the decision tree is called a confirmatory formulation test with human volunteers under in use conditions.

The SCCS emphasises that in the above tiered approach, a case-by-case study of every data set remains necessary as:
- the decision tree for hazard identification lacks a critical view on the applicability domain of the *in vitro* assays (e.g. exclusion of colourants and reducing substances, consideration of other endpoints, etc.).
- the decision tree for risk assessment includes a benchmark approach, which finally results in human safety testing. According to SCCNFP/0245/99, only compatibility testing in human volunteers is acceptable from an ethical point of view. It is the opinion of the SCCS that the weight of evidence needs to be carefully considered before such human testing is considered.

2) Mucous membrane irritation

Eye irritation tests have been developed to assess the production of changes in the eye following the application of a test substance to the anterior surface of the eye, which are fully reversible within 21 days of application. Eye corrosion is tissue damage in the eye, or serious deterioration of vision, following application of a test substance to the anterior surface of the eye, which is not fully reversible within 21 days of application.
There are presently no fully validated alternative methods replacing the classical Draize *in vivo* eye irritation test, which is now involving the use of one to three rabbits. Over the years, the test method has been subject to refinement and reduction measures, bringing the number of animals down from maximum six to maximum three, and involving a number of steps to be taken before the *in vivo* study can even be envisaged. These steps consist of:
- the evaluation of existing human and animal data;
- the analysis of structure activity relationships;
- a study of physicochemical properties and chemical reactivity (e.g. substances with a pH $\leq$ 2.0 or $\geq$ 11.5 will be considered as corrosive without *in vivo* testing);
- consideration of other existing information;
- taking into account the results from *in vitro* and *ex vivo* tests;
- the assessment of existing *in vivo* dermal irritancy or corrosivity data on the substance [EC B.5, OECD 405].

Although the animal study is still encountered in many ingredient dossiers, it forms part of the assays whose performance is affected by the earliest testing and marketing deadline of 11 March 2009. The available alternative methods for eye irritation / corrosion currently consist of a **screening battery of two assays**, **namely the BCOP** (Bovine Cornea Opacity Permeability) [OECD 437] **and the ICE** (Isolated Chicken Eye) [OECD 438]. They can be used in the process of hazard identification (not risk assessment) and enable to **eliminate severe eye irritants**, but fail to distinguish mild from non-irritants.
Together with the IRE (Isolated Rabbit Eye) and HET-CAM (Hen's Egg Test-Chorio Allantoic Membrane), they provide only supportive evidence for cosmetic ingredient safety assessment [SCCS/1294/10].
Several tests, including human reconstructed tissue models, are under validation, but these are not ready yet.

Finally, a number of cytotoxicity / cell function-based assays for water soluble substances underwent retrospective validation and peer review by ESAC [ESAC 2009c], but again, these methods need further critical evaluation before they can be considered full replacement methods for eye irritation [SCCS/1294/10].

In the light of the imposed testing ban on cosmetic ingredients, Colipa proposed a decision-tree approach for the integration of alternatives into tiered testing strategies for hazard and safety assessment of cosmetic ingredients and their use in products. It was acknowledged that, in contrast to the *in vitro* skin irritation tests, no single *in vitro* assay nor testing

battery has been validated as a full replacement for the rabbit Draize eye test. Nevertheless, two separate decision trees for eye irritation were put forward [McNamee et al. 2009]:

- A decision tree for hazard identification of the neat cosmetic ingredient, where physicochemical properties, read-across data, QSAR results and *in vitro* eye irritation data may lead to a classification of irritant or non-irritant. It is noted that the existing *in vitro* models may fail to identify non-irritants and weak to moderate eye irritants.
- A decision tree for risk assessment of the neat ingredient in its final formulation(s), where the measured formulation's eye irritancy in one or more *in vitro* eye irritation test(s) is to be compared against the measured irritancy of a benchmark control. The last step in the decision tree is called a confirmatory formulation test with human volunteers under in use conditions.

The SCCS emphasizes the fact that in the above tiered approach human safety testing for eye irritation is the final step in the risk assessment decision tree. The Committee considers that, **without the existence of a validated stand-alone *in vitro* test / testing battery, the tiered approach is too premature to be applied**. Human eye irritation testing may have serious health consequences for the volunteers involved.

Recently Scott et al. [2010] published the outcome of an ECVAM expert meeting (held in 2005), with the aim of identifying testing strategies for eye irritation. A hazard identification testing scheme was proposed using a bottom–up (starting with test methods able to accurately identify non-irritants) or top–down (starting with test methods able to accurately identify severe irritants) progression of *in vitro* tests. As such the approach intends to identify non-irritants and severe irritants, leaving all others to the (mild/moderate) irritant categories.
As identification of non-irritancy for the eye through *in vitro* methodology is today not yet possible, the practical value of the proposal is limited.

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)** [EC B.42, OECD 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.
   As far as the chemical field is concerned, the standard information requirements for the 1 tonne/year production level under REACH (Annex VII) mention the LLNA as the *first-choice method for in vivo sensitisation testing*. Only in exceptional circumstances, another test is considered appropriate and this can only be accepted when a justification is presented [2006/1907/EC].
   A reduced LLNA (rLLNA) was adopted by ESAC after a retrospective analysis of published data [Kimber et al. 2006]. However, as the rLLNA only uses the negative control group and the equivalent of the high-dose group of the original LLNA, no determination of the sensitising potency is possible. Therefore **the rLLNA is only suitable for screening purposes** to distinguish between sensitisers and non-sensitisers [SCCS/1294/10].

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.

In the field of alternative methods for skin sensitisation testing, several advances can be noted over the past years e.g. in the following areas:
- QSAR (quantitative structure-activity relationship) models, especially mechanism-based models;
- peptide reactivity assays where multiple parameters can be measured simultaneously (providing a more complete reactivity profile of the test substance);
- cell-based assays using different cell types;
- 3D-reconstituted skin models;

Although some of these assays show relatively high sensitivity and specificity for a number of sensitisers, their applicability in the risk assessment process still requires more time and efforts [Vandebriel and van Loveren 2010].

Recently cosmetic industry submitted to ECVAM a number of newly developed partial replacement *in vitro* tests for skin sensitisation. These were assessed to be ready to enter the formal validation process [SCCS/1294/10].

3-4.4 Dermal / percutaneous absorption

a. Major guidelines for 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, where 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 target compounds, occlusion, etc. [for review see Schaefer et al. 1996; ECETOC 1993; Howes et al. 1996].

The dermal / percutaneous absorption has been described by several international bodies [ECETOC 1993, US EPA 1996a, OECD 2004] using a wide variety of terms and it is recognised that confusion is possible. Therefore it seems appropriate to define some important terms in this particular field [SCCS/1358/10].

The **dermal / percutaneous absorption** process is a global term which describes the passage of compounds across the skin. This process can be divided into three steps:

- **penetration** is the entry of a substance into a particular layer or structure such as the entrance of a compound into the stratum corneum;
- **permeation** is the penetration through one layer into another, which is both functionally and structurally different from the first layer;
- **resorption** is the uptake of a substance into the vascular system (lymph and/or blood vessel), which acts as the central compartment.

Dermal / percutaneous absorption studies can be performed *in vivo* or *in vitro*. Today, however, *in vivo* dermal / percutaneous absorption testing is not an option any more for cosmetic ingredients in the European context, as the animal testing deadline of 11 March 2009 has passed [2003/15/EC].

Both *in vivo* and *in vitro* testing protocols form part of the lists of official EU and OECD test methods [EC B.44, 45; OECD 427, 428], accompanied by more detailed guidance on their performance [DG SANCO 2004, OECD 2004]. Whereas the first version of above-mentioned OECD Guideline 428 was issued in 2000, the SCCNFP already adopted its first set of basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients in 1999 [SCCNFP/0167/99]. This opinion, most recently updated in 2010 [SCCS/1358/10], focuses on the *in vitro* testing of cosmetic ingredients, whereas the general EU and OECD Guidance [DG SANCO 2004, OECD 2004] addresses percutaneous absorption from a much broader point of view by mentioning *in vivo* methods besides *in vitro* testing and by providing specifications for agricultural products and industrial chemicals besides cosmetics.

As a result, the SCC(NF)P/SCCS has always considered **a combination of the EU / OECD Guidelines and** its own "**Basic criteria**" **as essential for dermal / percutaneous absorption studies**.

b. The SCCS "Basic criteria"

The purpose of *in vitro* dermal absorption studies of cosmetic ingredients is to obtain qualitative and/or quantitative information on the substances that may enter, under in-use conditions, into the systemic compartment of the human body. The quantities can then be taken into consideration to calculate the margin of safety using the NOAEL of an appropriate repeated dose toxicity study with the respective substance.

In these relatively complex *in vitro* studies, there are a number of points that require special attention:

1) The design of the diffusion cell (technicalities and choice between static and flow through system).
2) The choice of the receptor fluid (physiological pH, solubility and stability of chemical in receptor fluid should be demonstrated, no interference with skin/membrane integrity, analytical method, etc.).
3) The skin preparations should be chosen and treated with care (human skin from an appropriate site remains the gold standard).
4) Skin integrity is of key importance and should be verified.
5) Skin temperature has to be ascertained at normal human skin temperature.
6) The test substance has to be rigorously characterised and should correspond to the substance that is intended to be used in the finished cosmetic products.
7) Dose and vehicle/formulation should be representative for the in-use conditions of the intended cosmetic product. Several concentrations, including the highest concentration of the test substance in a typical formulation, should be included.
8) Dose, volume and contact time with the skin have to mimic in-use conditions.
9) Regular sampling is required over the whole exposure period.
10) Appropriate analytical techniques should be used. Their validity, sensitivity and detection limits should be documented in the report.
11) The test compound is to be determined in all relevant compartments:
    - product excess on the skin surface (dislodgeable dose),
    - stratum corneum (e.g. adhesive tape strips),
    - living epidermis (without stratum corneum),
    - dermis,

- receptor fluid.

12) Mass balance analysis and recovery data are to be provided. The overall recovery of test substance (including metabolites) should be within the range of 85-115%.

13) Variability / validity / reproducibility of the method should be discussed. The SCCS considers that for a reliable dermal absorption study, 8 skin samples from at least 4 donors should be used.

The amounts measured in the dermis, epidermis (without stratum corneum) and the receptor fluid will be considered as dermally absorbed and taken into account for further calculations.

When studies correspond to all of the basic requirements of the SCCS, the **mean + 1SD** will be used for the calculation of the MoS. The reason for not using the mean *per se* is the frequently observed high variability in the *in vitro* dermal absorption assays. In case of significant deviations from the protocol and/or very high variability, the **mean + 2SD** will be used as dermal absorption for the MoS calculation[1].
In case the results are derived from an inadequate *in vitro* study, **100%** dermal absorption is used. However, in case MW > 500 Da and log P_{ow} is smaller than -1 or higher than 4, the value of **10%** dermal absorption is considered.

3-4.5 Repeated dose toxicity

Repeated dose toxicity comprises the adverse general toxicological effects (excluding reproductive, genotoxic and carcinogenic 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: repeated dose 90-day dermal toxicity study using rodent species [EC B.28, OECD 411]
   - Sub-chronic inhalation toxicity study: repeated dose 90-day inhalation toxicity 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. Preferably studies of 90 days or more should be used in safety assessments. If studies of only 28 days duration are available, an additional uncertainty factor can be used in the calculation of the MoS [EChA 2008b].
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.

In a number of cases dermal repeated dose toxicity studies are present among the submitted data. This could for example be the case for a UV-filter (in USA and Canada

[1] This pragmatic approach was established after in-depth discussions in a special working group with all parties involved.

considered to be a drug and as such generally tested via the dermal route). These studies are taken into consideration by the SCCS.

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.

For repeated-dose toxicity testing, **currently no validated or generally accepted alternative method is 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 [SCCS/1294/10].

In the original notification process of dangerous substances, repeated dose toxicity studies were required when the substance under consideration was produced or imported in amounts exceeding 1 tonne/year [92/32/EEC]. Under REACH, this threshold was raised to 10 tonnes/year [2006/1907/EC].
In the case of the development of cosmetic ingredients which have specific biological properties and which will be in contact with human skin for a long period of time, the SCCS 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 limited requirements imposed by REACH [2006/1907/EC].

Therefore **the SCCS 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

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 gene segment, a block of genes or chromosomes. The term clastogenicity is used for agents giving rise to structural chromosome aberrations. A clastogen can cause breaks in chromosomes that result in the loss or rearrangements of chromosome segments. Aneugenicity (aneuploidy induction) refers to the effects of agents that give rise to a change (gain or loss) in chromosome number in cells. An aneugen can cause loss or gain of chromosomes resulting in cells that have not an exact multiple of the haploid number [2006/1907/EC].

Genotoxicity is a broader term and refers to processes which alter the structure, information content or segregation of DNA and are not necessarily associated with mutagenicity. Thus, tests for genotoxicity include tests which provide 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, as well as tests for mutagenicity [2006/1907/EC, EChA 2008a].

As a general recommendation, the SCCS is of the opinion that the base level of evaluation of the potential for mutagenicity of a cosmetic ingredient to be included in Annexes III, IV, VI and VII of Council Directive 76/768/EEC should include tests to provide information on the three major genetic endpoints, namely 1) mutagenicity at a gene level, 2) chromosome breakage and/or rearrangements (clastogenicity), and 3) numerical chromosome aberrations (aneugenicity): these three base levels of information represent the actual consensus of international groups of scientific experts [Muller et al. 2003, Dearfield et al. 2002,

2006/1907/EC], and of an expert advisory committee [COM 2000, 2010]. Several well-established *in vitro* mutagenicity/ genotoxicity tests are available, described in OECD Guidelines[1] and/or in Regulation (EC) No 440/2008 [2008/440/EC]. The SCCS is of the opinion that for this task only *in vitro* genotoxicity tests which measure a real mutation endpoint (gene or chromosome mutations) are qualified; so-called indicator tests which do not measure irreversible DNA damage should not be used. Moreover, the SCCS recommends before undertaking any testing, a thorough review of all available (literature) data on the compound under study, including its chemistry, toxicokinetic and toxicological profile, as well as data on analogous ingredients.

In principle, the SCCS recommends for the base level testing of cosmetic ingredients, three assays, represented by the following test systems:

1. Tests for gene mutation:
   - i) Bacterial Reverse Mutation Test [EC B.13/14, OECD 471]
   - ii) *In Vitro* Mammalian Cell Gene Mutation Test [EC B.17, OECD 476]
2. Tests for clastogenicity and aneugenicity
   - i) *In Vitro* Micronucleus Test [OECD 487]

A caveat to the use of the existing *in vitro* tests is the relatively high rate of unexpected negative (negative for carcinogens) and, particularly, unexpected positive (positive for non-carcinogens) results. An evaluation by Kirkland et al. [2005] for combinations of two or three assays, demonstrated that with an increase in the number of tests, the number of unexpected positives increases whereas the number of unexpected negatives decreases. It was argued that a strategy of 3 tests might not be better, although generally considered as "safer".

A topic for further discussion is a recent analysis of Kirkland et al., showing that the sensitivities of the 2- and 3-test batteries seem quite comparable when an existing database of rodent carcinogens and a new database of *in vivo* genotoxins, together over 950 compounds, are considered. Using data from the gene mutation test in bacteria and the *in vitro* micronucleus test, appear to allow the detection of all relevant *in vivo* carcinogens and *in vivo* genotoxins for which data exists in these databases [Kirkland et al. 2011]. The combination of these two assays would cover the three endpoints, as the *in vitro* micronucleus assay detects both structural and numerical chromosome aberrations.

Discussions are going on whether it is justified to incorporate 2 instead of 3 *in vitro* assays as indicated above. Independent from this debate, there may be instances for which the basic requirement should be modified: in these cases a scientific justification for the deviation and the decision taken should be given.
For some classes of compounds with specific structural alerts, it is established that specific protocol modifications/additional tests are necessary for optimal detection of genotoxicity.

If equivocal or inconclusive results are obtained which make a final decision on the genotoxicity impossible, further testing may be performed, e.g. the *in vitro* chromosome aberration test [EC B.10, OECD 473].

A number of alternative methods are under development but have not yet been validated. It is not excluded that these could add to a weight of evidence approach. Examples are:
- the Micronucleus test in reconstructed human skin
- the Comet assay in reconstructed human skin

[1] http://oberon.sourceoecd.org/vl=8055337/cl=27/nw=1/rpsv/cw/vhosts/oecdjournals/1607310x/v1n4/ contp1-1.htm, consulted September 2010

Cells should be exposed to the test substance both in the presence and absence of an appropriate metabolic activation system. The most commonly used system is a cofactor-supplemented S9-fraction prepared from the livers of rodents (usually rat) treated with enzyme-inducing agents such as Aroclor 1254 or combination of phenobarbital and β-naphthoflavone. The choice and concentration of a metabolic activation system may depend upon the class of chemical being tested. In some cases it may be appropriate to utilise more than one concentration of S9-mix. For azo dyes and diazo compounds, using a reductive metabolic activation system may be more appropriate [Matsushima 1980; Prival et al. 1984].

In cases where clearly negative results are seen in the conducted tests, a mutagenic potential is excluded. In cases where a clear positive result is seen in one of the tests, the compound has to be considered as a (*in vitro*/intrinsic) mutagen. Under the testing/marketing ban of the 7th amendment of the Cosmetics Directive [2003/15/EC] on cosmetic ingredients, further *in vivo* testing to confirm or, predominantly, to overrule the positive *in vitro* findings is no longer possible. Unfortunately, at present no validated methods are available that allow the follow-up of positive results from standard *in vitro* assays [SCCP/1212/09].
The SCCS is of the opinion that with the *in vivo* testing ban for cosmetic ingredients, the safety of many potential new cosmetic ingredients cannot be assessed.

Under the Colipa genotoxicity program, currently two projects are ongoing: one with the aim of improving the existing *in vitro* standard genotoxicity assays so that the number of false positive results (denominated as 'unexpected' positives) can be reduced and a second one to develop new *in vitro* assays that may serve as follow-up of positive results from the standard battery. In addition, a workshop was held to determine the best way of using the currently available methods and approaches to enable a sound assessment of the genotoxic hazard of cosmetic ingredients.
It resulted in the publication of a decision tree, which starts with the gathering of physicochemical data, QSAR analysis and the performance of a classic Ames test, and finally results in the incorporation of genotoxicity endpoints in repeated dose toxicity tests (allowed until 11 March 2013) [Pfuhler et al. 2010]. This approach has not been accepted by the SCCS.

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 the following ***in vivo* assays**:

1) Carcinogenicity test [EC B.32, OECD 451]
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. The *in vitro* Cell Transformation Assays (CTA's) may detect both genotoxic and non-genotoxic carcinogens. These tests are at present under ECVAM validation, but the results are not yet available [Farmer 2002, Hayashi et al. 2008, Van Benthem et al. 2010].

As far as genotoxic compounds are concerned, *in vitro* mutagenicity tests are quite well developed. Tests for detecting non-genotoxic carcinogens, however, are not available. 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 Regulation (EC) No 440/2008 [2008/440/EC].

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 Embryonic 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 might be useful in the CMR strategy for screening out embryotoxic substances. However, as **the applicability domain of these 3 alternative embryotoxicity tests is under discussion** [Marx-Stoelting et al. 2009], they cannot yet be used for quantitative risk assessment. The **EST can be considered as a screening test** and further research remains necessary.

The endpoint of reproduction toxicity is not covered by the above systems. No alternative methods are available in this area.

To this respect, it can be stated that several *in vitro* methodologies, each covering one of the three biological components of the reproductive cycle (male & female fertility, implantation and pre- and postnatal development), were developed under the EU 6th Framework project ReProTect[1]. The tests reflect various toxicological mechanisms such as effects on Leydig and Sertoli cells, folliculogenesis, germ cell maturation, motility of sperm cells, steroidogenesis, the endocrine system, fertilisation, and on the pre-implantation embryo. Nevertheless, much more information and research remain needed before regulatory acceptance can be envisaged.

3-4.9 Toxicokinetic studies

The term "toxicokinetic studies" is in the context of chemical substances such as cosmetic ingredients, 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].

[1] http://www.reprotect.eu/, consulted December 2010

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.

Only in a limited number of cases human toxicokinetic study results were available for cosmetic ingredients, e.g. p-phenylenediamine and 4-methyl benzylidene camphor, to address specific questions with respect to human safety [SCCP/0989/06, SCCP/1184/08].

In the context of the EU cosmetic legislation, a review of the actual status of alternatives to animal toxicokinetic studies was recently carried out by a group of experts in the field [Pelkonen et al. 2010]. They came to the conclusion that some important gaps still exist. As toxicokinetic data are important in extrapolating both *in vitro* and *in vivo* animal data to man, a lot of efforts will be needed to make substantial progress in this field.

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 UV/visible light.

In 1998, the SCCNFP recommended the use of this *in vitro* method for the determination of the phototoxicological/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 Directive 67/548/EEC (recently replaced by Regulation (EC) No 440/2008) [EC B.41, OECD 432], making its use mandatory for testing for phototoxic potential.

The reliability and relevance of the *In vitro* 3T3 NRU Phototoxicity Test was 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 [EC B.41].

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 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.

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

The previous version of the Notes of Guidance [SCCNFP/0690/03] already mentioned that for the detection of photochemical clastogenicity/mutagenicity several assays had been adapted to a combined treatment of chemicals with Ultraviolet-Visible (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].

Meanwhile, the 2004 state of the art of the existing principles and test methods in the field of photomutagenicity / photogenotoxicity is summarised in a review of Brendler-Schwaab et al., which was the report of the Gesellschaft für Umweltmutationsforschung (GUM) Task Force on photochemical genotoxicity. The methods described include the photo-Ames test, the photo HPRT / photo-mouse lymphoma assay, the photo-micronucleus test, the photo-chromosome aberration test and the photo-Comet assay.

For each method, the results of compounds tested are briefly summarised from the available literature. One of the authors' conclusions is that, in many cases, the concurrent use of irradiation while performing a classical mutagenicity / genotoxicity study, does not significantly alter the existing OECD protocol without irradiation. Therefore they consider the majority of the described photomutagenicity / photogenotoxicity tests as being valid [Brendler-Schwaab 2004].

Taking the GUM Task Force results into consideration, the SCCS evaluates the individual photomutagenicity/photogenotoxicity tests and their scientific value on a case-by-case basis, keeping in mind the general provisions for the classical mutagenicity/genotoxicity testing battery as mentioned in 3-4.6.

Considering the above and also referring to a recent discussion paper by EMEA [EMEA 2009], it is clear that the validity of photogenotoxicity testing is increasingly being questioned.

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 the human situation. 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 no concern is raised. 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 SCCNFP/0068/98 and SCCNFP/0245/99.

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 SCCS 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.

3-5 TOXICOLOGICAL REQUIREMENTS FOR INCLUSION OF A SUBSTANCE IN ONE OF THE ANNEXES TO DIRECTIVE 76/768/EEC (EVALUATED BY THE SCCS)

3-5.1 General toxicological requirements

When a cosmetic ingredient dossier is submitted for evaluation by the SCCS, 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 is subjected to ethical concerns. This is even more the case for the predictive testing of potentially cutaneous sensitising cosmetic ingredients or mixtures of ingredients. A risk for the 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 Regulation (EC) No 440/2008 [2008/440/EC], 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 considering the conduct of animal studies, not only the imposed testing and marketing bans in the cosmetic legislation need to be kept in mind, but equally Art.7 of Council Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes. The latter states that *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*. This basic principle is further developed in Art.4 of the revision of Directive 86/609/EEC, which literally introduces the 3R-principle into the European legislation [2010/63/EU]. It will completely repeal Directive 86/609/EEC in May 2013.

It should be further noted that:

- **Whenever 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 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.
- Ensuring that files for evaluation are **complete** when submitted is an important requirement. 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/mixture;
  - 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.
- 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 Directive 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 not allowed to be used in cosmetic products, unless 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 authorised concentration in the finished product or where certain applications need to be restricted. The general requirements as defined in 3-5.1 apply for the inclusion of a cosmetic ingredient in this Annex, unless it belongs to the category of hair dyes or hair dye components (see 3-8).
Part 1 of Annex III contains a number of oxidative (permanent) and non-oxidative hair dye components, such as 1-naphthol, resorcinol and many others (ref. n°16, 22, 189-205, 208-209, ...); while Part 2 provides provisional authorisation for 29 colouring agents used as oxidative and/or non-oxidative hair dye components.

3-5.4 Annex IV

Annex IV constitutes a list of colouring agents permitted for use in cosmetic products.

A number of these colorants 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 colorants do not differ from those defined in 3-5.1, unless they are being used as hair dyes or hair dye components (see 3-8).

3-5.5 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.

3-5.6 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 by using the methodologies as described in section 3-4.10.
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.

Finally, it must be emphasised that 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], and that photostability data under conditions of use should be provided.

3-5.7 Requirements for partial evaluations

In some cases, either upon request of the SCCS 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, however, may not be adequate to answer the question of the new risk. Therefore, complete dossiers may be required, even though a re-evaluation of only a part of a dossier appears 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 majority of the opinions of the SCCS concerns the safety assessment of ingredients taken up in the Annexes to Directive 76/768/EEC, some general considerations apply to all other ingredients.

Since cosmetic ingredients are in principle also chemical substances, a number of these compounds were at a certain time notified as dangerous substances in the EU in order to comply with the requirements of the chemical legislation (Dangerous Substances legislation, previously Directive 67/548/EEC, now EC Regulation N° 2006/1907/EC). For such compounds the required data package is mainly triggered by their produced / EU imported

annual volumes. The fact that some of these substances will also be used as cosmetic ingredients, however, does not trigger any additional toxicological data requirement.

The toxicological requirements for dangerous substances newly produced /EU imported at levels between 1 and 10 tonnes per year (a category to which several cosmetic ingredients belong), in most cases 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 [2006/1907/EC].

A scientifically sound safety evaluation, based on less data than those mentioned above for the 1 -10 tonnes/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 concentrations.

Therefore, it would be very useful if, in analogy with the ingredients taken up in the Annexes to Directive 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 elaborated data packages are available (e.g. high production volume chemicals), a large number of the general requirements described in 3-5.1 should be covered.

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 and/or other additives added.

b) Complex ingredients of animal origin

- species (bovine, ovine, crustacean, …)
- 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,…
- physical and chemical specifications
- microbiological quality including relevant viral contamination
- additional external contamination
- preservatives and/or other additives 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 and/or other additives 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 and/or other additives 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 SCCS 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 the ingredients which 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 SCC(NF)P opinions [SCCNFP/0017/98, SCCNFP/0392/00, SCCNFP/0450/01, SCCNFP/0770/03, SCCNFP/0771/03, SCCP/1023/06]; a confirmation that all legally binding 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.
Specific labelling to reduce the incidence of contact-allergic reactions in fragrance-sensitive consumers has been foreseen by the inclusion of 26 potentially sensitising fragrance ingredients in Annex III to Directive 76/768/EEC. More specifically, the presence of these substances must be indicated in the list of ingredients on the label when their concentrations in the final product exceed 0.001 % in leave-on products or 0,01 % in rinse-off products [2003/15/EC].

3-6.4 Potential endocrine disruptors

Chemical substances with a potential to modulate the hormonal system, may be expected to have harmful effects on human or animal health, if they are included in cosmetic products or released to the environment.
The so-called endocrine disrupting chemicals (EDCs) have been subject to intensive scientific investigation and discussion since the 1990s [Damstra et al. 2002, Hotchkiss et al. 2008], and several working **definitions** have been suggested.
The SCCS, in accordance with the European Commission[1], endorses the following WHO/IPCS definitions [Damstra et al. 2002]:

> *"A **potential endocrine disruptor** is an exogenous substance or mixture that possesses properties that might lead to endocrine disruption in an intact organism or its progeny, or (sub)populations."*

and

[1] http://ec.europa.eu/environment/endocrine/definitions/endodis_en.htm, consulted December 2010

*"An **endocrine disruptor** (ED) is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations".*

The OECD is currently developing guidance documents and (enhanced) testing guidelines for these substances[1].
Evidence for a hormone-like activity in screening assays would already categorize a substance as a **potential ED** (hazard, suspicion category) which might be confirmed by activity in *in vivo* assays on endocrine activity. *In vivo* assays rank very high, both in early and later stages of tiered testing [Gelbke et al. 2004; Gelbke et al. 2007; Hotchkiss et al. 2008]. However, a categorization as **ED** requires adverse toxicological effects as a result of standardized animal experiments according to OECD guidelines, *e.g.* on developmental and reproductive toxicity [Degen and Owens 2008] from which a risk can be derived. In the light of the different levels of information, it is important to make the above distinction, especially in a regulatory setting. In this context, the SCCS also points to the quote: 'Endocrine disruption is not considered a toxicological endpoint *per se* but a functional change that may lead to adverse outcomes' [Damstra 2002].
The main area of concern for *adverse* outcomes initially was reproductive and developmental toxicity due to interference with the *sex hormone system*. Focus was on chemicals which mimic or antagonize the action of endogenous estrogens and/or androgens [Hotchkiss et al. 2008]. Now, there is also some concern on potential disruption of the thyroid hormone system (due to its role in development), and on the immune and neuro-endocrine system. These mechanisms, however, only cover a small part of the complex endocrine system[2].

In 2000, the European Commission (Directorate-General for the Environment) issued a document titled "Towards the establishment of a priority list of substances for further evaluation in their role in endocrine disruption" [DG ENV 2000]. Initially, a working list of 564 substances was drawn up for which information on potential endocrine disrupting effects had been gathered in four steps: (1) a review of existing lists and other sources of information, (2) selection of highly persistent and/or high production volume (HPV) chemicals, (3) a preliminary evaluation of scientific evidence of endocrine disrupting effects and (4) a preliminary evaluation of exposure to humans and wildlife. The results of this and further refinements, resulting from a review process determining the strength of evidence for endocrine disruption, were compiled in a database[3].

However, it is important that the **listings** produced are **not** regarded as **final**.
From the 564 chemicals that were suggested as being suspected EDs, 147 were considered likely to be either persistent in the environment or produced at high volumes. Of these, however, in a first assessment clear evidence of endocrine disrupting activity was noted for only 66 (assigned Category 1 using the criteria adopted in the study). A further 52 chemicals showed some evidence suggesting potential activity (Category 2). In total 118 substances were categorised in the first exercise of priority setting. Of the 66 chemicals in Category 1, humans were considered likely to be exposed to 60 of them[4].

There is not yet a harmonized approach on health risk assessment procedures for (potential) endocrine disrupting compounds within the different regulatory frameworks in the EU or internationally [Beronius et al. 2009, Harvey and Everett 2006].
A draft concept paper on the development of a stepwise procedure for the assessment of substances with endocrine disrupting properties according to the plant protection products regulation [2009/1107/EC] was the outcome of a recent workshop on that topic hosted by the

1 http://www.oecd.org/document/62/0,2340,en_2649_34377_2348606_1_1_1_1,00.html, consulted December 2010
2 http://ec.europa.eu/environment/endocrine/definitions/affect_en.htm, consulted December 2010
3 http://ec.europa.eu/environment/endocrine/strategy/short_en.htm, consulted December 2010
4 http://ec.europa.eu/environment/endocrine/strategy/substances_en.htm, consulted December 2010

BfR in Berlin. It was proposed to categorize chemicals according to their toxicological potency[1].

Chemical substances with endocrine disrupting properties have been addressed also in the new European legislation for existing and new industrial chemicals under REACH. The European Chemicals Agency (ECHA) and the European Food Safety Authority (EFSA) are developing a concept for the assessment of **potential ED** in line with the OECD conceptual framework for potential endocrine disruptors.

Potential endocrine disruptors as cosmetic ingredients:

In 2001, ingredients of cosmetic products were first mentioned as **potential ED**, *i.e.* a number of UV filters present in sun protection products which displayed estrogenic effects *in vitro* and *in vivo* in mice [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. Moreover, the *in vitro* potency of the UV filters studied was not only considerably lower 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 steroids used in 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 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].

Since then other potential endocrine disruptors present in cosmetics have been reviewed by SCCP and SCCS, *i.e.* parabens [SCCP/1017/06, SCCP/1183/08], homosalate [SCCP/1086/07], triclosan [SCCP/1192/08], and cyclomethicone [SCCS/1241/10]. These opinions came to the conclusion that endocrine/hormonal activities were not the critical endpoint for assessing the safety of these substances. Nonetheless, these opinions illustrate the types of *in vitro* studies suitable to detect different hormonal activities (**potential ED**) and *in vivo* studies relevant for detection of related developmental and reproductive toxicity. Thereby these opinions provide some guidance on the types of data needed in a scientific evaluation of substances with respect to endocrine disrupting properties.

For cosmetic ingredients it might be impossible in the future to differentiate between **potential ED** and **ED** due to the upcoming ban on animals testing in 2013 which has been pointed out in recent documents. They acknowledge the fact that the replacement of animal test methods by alternative methods in relation to complex toxicological endpoints remains scientifically difficult, despite the additional efforts launched at various levels [SCCS/1294/10, JRC 2010].

3-6.5 Animal-derived ingredients, incl. 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.
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 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) [SCCNFP/0521/01].
The most recent adaptation to entry n° 419 in Annex II of Directive 76/768/EEC was issued in March 2007 [2006/78/EC] and resulted in:

[1] http://www.bfr.bund.de/cm/289/development_of_a_stepwise_procedure_for_the_assessment_of_substances_with_endocrine_disrupting_properties.pdf, consulted December 2010

"**419.** Category 1 material and Category 2 material as defined in Articles 4 and 5 respectively of Regulation (EC) No 1774/2002 of the European Parliament and of the Council (*), and ingredients derived therefrom."

(*) OJ L 273, 10.10.2002, p. 1

As indicated, 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.

Finally, taking into account EC Regulation No 1774/02 laying down health rules concerning animal by-products not intended for human consumption, the SCCP was of the opinion that ingredients derived from category 1 (*inter alia* specific risk material) and category 2 (*inter alia* 'fallen stock') material raise concern in terms of biological risk for human health and therefore must not be present in cosmetic products. Since category 3 material is defined as being fit for human consumption, it may also be used as cosmetic ingredient [SCCP/0933/05].

3-6.6 CMR-ingredients

In September 2001, the SCCNFP issued its first opinion on substances officially classified as carcinogenic, mutagenic or toxic to reproduction (CMR) [SCCNFP/0474/01]. The Committee proposed the prohibition of the intentional use in cosmetic products of CMR substances category 1 or 2 and substances with similar potentials (except substances only carcinogenic by inhalation). The same was proposed for CMR category 3 substances **unless** it could be demonstrated that their levels did not pose a threat to the health of the consumer. If a CMR substance was present in a cosmetic product from its presence in a natural ingredient, as an impurity, or because it was formed during manufacturing, it needed to be demonstrated that the product did not pose a threat to the health of the consumer.

The SCCNFP opinion on CMR substances was translated into the cosmetics' legislation through the 7th Amendment [2003/15/EC] and resulted in the gradual uptake of all concerned CMR substances in Annex II to the Cosmetic Products Directive.

The new EU Regulation on classification, labelling and packaging of substances and mixtures [1272/2008/EC] brings the CMR classification in line with the UN GHS[1] terminology.
The chemical legislation classifies substances that are *carcinogenic, germ cell mutagenic or toxic for reproduction* in respectively *Category 1A, 1B and 2*, under part 3 of Annex VI to Regulation (EC) N°1272/2008. It is mainly a question of nomenclature, as the basis for the classification into the three different categories generally remains unaltered (see Table 1).

The Recast of the Cosmetic Products Directive makes use of the new nomenclature as stated in Table 1 and partially retains the original CMR provisions. Nevertheless it also introduces an important derogation to the ban by the additional provision for CMR Cat. 1A or 1B substances which (1) comply with the European food safety requirements[2], (2) cannot be replaced by suitable alternatives, (3) have a well-defined use pattern and known exposure and (4) were evaluated and found safe by the SCCS for the intended use in cosmetic products [2009/1223/EC]. These substances could be allowed to be used as cosmetic ingredients within Europe under specific conditions.

1 http://www.unece.org/trans/danger/publi/ghs/ghs_welcome_e.html, consulted December 2010

2 As defined in Regulation (EC) No 178/2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety

Table 1: Changes in CMR nomenclature due to the adaptation of the EU classification and labelling system to the UN GHS principles.

Category according to 'old' classification system [Directive 2001/59/EC]	Category according to 'new' classification system [Regulation N° 1272/2008]
Carcinogen Cat. 1 *Substance known to be carcinogenic to man*	**Carcinogen Cat. 1A** *Known to have carcinogenic potential for humans*
Classification largely based on human evidence: causal association between exposure and development of cancer.	
Carcinogen Cat. 2 *Substance that should be regarded as if it is carcinogenic to man*	**Carcinogen Cat. 1B** *Presumed to have carcinogenic potential for humans*
Classification largely based on animal evidence of carcinogenic effects or case-by-case scientific judgment based upon studies showing limited evidence of carcinogenicity in humans together with sufficient evidence in experimental animals.	
Carcinogen Cat. 3 *Substance that causes concern for man owing to possible carcinogenic effects*	**Carcinogen Cat. 2** *Suspected human carcinogen*
Classification largely based on animal evidence; e.g. studies showing limited evidence of carcinogenicity in humans together with limited evidence in experimental animals.	
Mutagen Cat. 1 *Substance known to be mutagenic to man*	**Germ cell mutagen Cat. 1A** *Substance known to induce heritable mutations in the germ cells of humans*
Classification based on human evidence: positive evidence of human epidemiological studies.	
Mutagen Cat. 2 *Substance that should be regarded as if it is mutagenic to man*	**Germ cell mutagen Cat. 1B** *Substance to be regarded as if it induces heritable mutations in the germ cells of humans*
Positive result(s) in *in vivo* heritable germ cell mutagenicity tests or in somatic *in vivo* mutagenicity tests, the latter in combination with evidence that the substance has potential to cause mutations to germ cells.	
Mutagen Cat. 3 *Substance that causes concern for man owing to possible mutagenic effects*	**Germ cell mutagen Cat. 2** *Substance which causes concern for humans owing to the possibility that it may induce heritable mutations in the germ cells of humans*
Evidence from *in vivo* and in some cases from *in vitro* somatic cell mutagenicity tests.	
Toxic to reproduction Cat. 1 *Substance known to impair fertility or to cause developmental toxicity in humans*	**Reproductive toxicant Cat. 1A** *Known human reproductive toxicant*
Classification largely based on human evidence: causal association between exposure and adverse effect on sexual function and fertility, or on development.	
Toxic to reproduction Cat. 2 *Substance that should be regarded as if it impairs fertility or causes developmental toxicity in humans*	**Reproductive toxicant Cat. 1B** *Presumed human reproductive toxicant*
Classification largely based on animal evidence: clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, unless the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects.	
Toxic to reproduction Cat. 3 *Substance that causes concern for human fertility or that causes concern for humans*	**Reproductive toxicant Cat. 2** *Suspected human reproductive toxicant*

owing to possible developmental toxic effects	
Classification largely based on animal evidence; limited evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects.	

3-6.7 Nanomaterials

A **nanoparticle** is considered *a particle with one or more dimensions at the nanoscale and is* thus defined as *a particle with at least one dimension <100nm. A* ***nanomaterial*** *is a material with one or more external dimensions, or an internal structure, on the nanoscale, which could exhibit novel characteristics compared to the same material without nanoscale features. Two principal factors cause the properties of nanomaterials to differ significantly from bulk materials: increased relative surface area, and quantum effects* [SCCP/1147/07].

In its plenary meeting of September 2005, the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) adopted an opinion on the appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies [SCENIHR/002/05]. A number of other reviews have since concluded that the existing risk assessment paradigm, in use for conventional chemicals, should in principle be applicable to engineered nanoparticles. However, it has also been pointed out that the **current testing methods may need certain adaptations** to take account of the special features of nanoparticles [Rocks et al. 2008, SCENIHR 2009, OECD 2009]:

- Because of high surface energies, nanoparticles tend **to stick together** to form larger agglomerates and aggregates, or bind with other moieties. Depending on the test medium, this may lead to substantial **changes in the applied concentration** of a nanoparticle during the test, and may affect the results. Characterisation of nanoparticles, prior to and during a test, is therefore key to ensure that valid results are obtained.
- Some test methods are suitable for substances that are soluble at more than 1 mg/l. Testing of insoluble nanoparticles must therefore take into account that they may be present in the test medium as a **nano-suspension rather than a solution**. The applied concentration of a nanoparticle may drop during a test due to sedimentation, binding with other moieties in the medium, or sticking to sides of the glass/plastic ware. This requires **ascertaining the stability** of a nano-suspension to ensure that the applied concentration of a nanoparticle is maintained during the test.
- Nanoparticles are also known to **adsorb or bind different substances on their surfaces**, including proteins [Šimon and Joner 2008, Lynch and Dawson 2008]. They may bind different substances in the test medium and carry them into the exposed test systems, which may lead to artefacts. Again characterisation of nanoparticles, and the use of appropriate controls, should be ensured so that a test does not generate erroneous results.
- The toxicological hazards of chemical substances are measured and expressed in weight or volume units (such as mg/kg, or mg/l). These **conventional metrics may not be appropriate** for nanoparticles due to large aspect ratios. Discussions around identification of appropriate dose metrics for nanoparticle are currently ongoing. Until suitable parameters are identified, it is important that tests on nanoparticles are evaluated using different dose-describing parameters, such as weight/volume concentration, particle number concentration, specific surface area etc.
- The ability of nanoparticles (especially in the lower nm range) to **penetrate cellular membrane barriers** adds another dimension to particulate toxicology. Currently, there are uncertainties over whether the endpoints identified under the current testing methods will be sufficient to identify and characterise all the hazards that may be associated with a nanoparticle. These issues are under discussion by the SCCS with a focus on the safe use of nanoparticles in cosmetic products. An opinion in this regard will follow in due course.
- The most recent EU cosmetic legislation [2009/1223/EC] foresees that cosmetics containing nanomaterials require special attention. They will **need to be notified to the**

Commission 6 months prior to placing on the market and some nanomaterial **specific information** (e.g. particle size, physical and chemical properties, toxicological profile and exposure & risk assessment, ...) needs to be provided for any nano-sized cosmetic ingredient not (to be) taken up in the Annexes of the Regulation. In case the Commission has concerns regarding the safety of a nanomaterial, an **SCCS opinion** shall be required.

- As a final remark, the SCCS emphasizes that nanomaterials are currently **not used as** reference compounds **for the validation of alternative methods**.

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

BMD(L): The Benchmark Dose (BMD) is proposed as an alternative for the classical NOAEL and LOAEL values. The BMD is based on a mathematical model being fitted to the experimental data within the observable range and estimates the dose that causes a low but measurable response (the benchmark response BMR) typically chosen at a 5 or 10% incidence above the control. The BMD lower limit (BMDL) refers to the corresponding lower limits of a one-sided 95% confidence interval on the BMD.

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 EC B.26].

Dosage: A general term comprising of dose, its frequency and duration [EC B.26]. In the calculations of the Margin of Safety, dosage is expressed in mg/kg body weight/day.

NO(A)EL: The No Observed (Adverse) Effect Level is the outcome of repeated dose toxicity studies, such as 28-day or 90-day tests with rats, mice, rabbits or dogs, chronic toxicity tests, carcinogenicity tests, teratogenicity tests, reproduction toxicity tests, etc. It is the highest dosage for which no (adverse) effects can be observed [based on EC B.26].
In the calculation of the MoS, the lowest obtained NO(A)EL value is used, in order to take into account 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.

LO(A)EL: The Lowest Observed (Adverse) Effect Level is the outcome of long-term toxicity studies, such as 28-day or 90-day tests with rats, mice, rabbits or dogs, chronic toxicity tests, carcinogenicity tests, teratogenicity tests, reproduction toxicity tests, etc. It is the lowest dosage where (adverse) effects can be observed. In the calculation of the MoS, the lowest obtained LO(A)EL value is used when a NO(A)EL is not available. The LO(A)EL should be expressed as mg/kg bw/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).

TD_{50}: The TD_{50} is defined as the chronic dosage rate (in mg/kg bw per day) which, for a given target site(s), would cause tumours in half of the animals within some standard experimental time – the "standard lifespan" for the species. A TD_{50} can be calculated either for a particular category of neoplastic lesion (e.g. malignant tumours only, liver tumours only) or for all tumours.

3-7.2 Calculation of the Margin of Safety of a cosmetic ingredient

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 and it is calculated by dividing the lowest NO(A)EL value of the cosmetic ingredient under study by its estimated SED:

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

The above equation is made up by three important parameters:

a) The Margin of Safety (MoS)

The 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.2). The **WHO proposes a minimum value of 100**, and it is generally accepted that the **MoS should at least be 100 to declare a substance safe for use**.

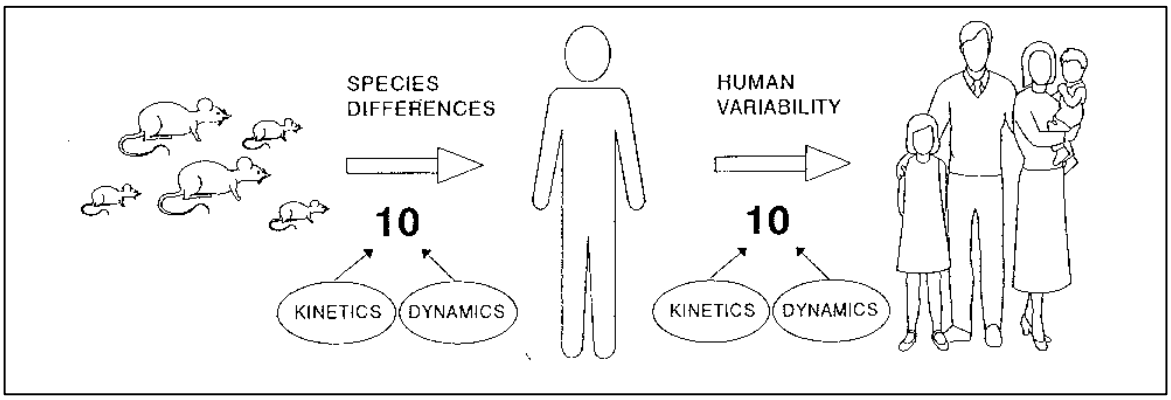

Fig.2: Schematic representation of the extrapolation from animal to man [Renwick, 1998].

As shown in Fig.2 the value of 100 consists of a factor 10 for the extrapolation from animal to man and another factor 10 taking into account the inter-individual variations within the human population. These factors can be further subdivided as indicated in Fig.3.
With regard to rounding and number of digits given for the MoS, this should be based on the precision of the underlying data. The biological variability of toxicity data *in vivo* generally is > 10%. The indication of more than 2 digits in the final MoS is therefore not recommended.

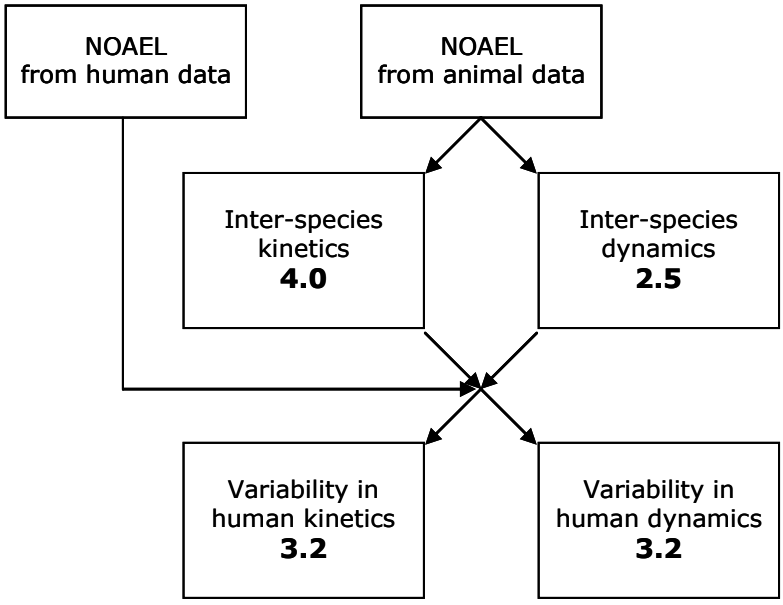

Fig.3: Further subdivision of the Margin of Safety, taking into account kinetics and dynamics [based on WHO, 1994].

b) The NO(A)EL value

The No Observed (Adverse) Effect Level is defined as the highest dose or exposure level where no (adverse) treatment-related findings are observed. It is mainly derived from repeated dose animal studies (90 day, developmental toxicity studies, etc).
As far as the determination of critical effects in repeated dose toxicity studies is concerned, the available repeated dose toxicity data should be evaluated in detail for a characterisation of the health hazards upon repeated exposure. In this process an assessment of all toxicological effect(s), their dose-response relationships and possible thresholds are taken into account. The evaluation should include an assessment of the severity of the effect, whether the observed effect(s) are adverse or adaptive, whether the effect is irreversible or not or whether it is a precursor to a more significant effect or secondary to general toxicity. Correlations between changes in several parameters, e.g. between clinical or biochemical measurements, organ weights and (histo)pathological effects, will be helpful in the evaluation of the nature of effects. Further guidance to this issue can be found in several publications [WHO 1994, WHO 1999, ECETOC 2002, EChA 2008a].

When making use of the **Lowest Observed (Adverse) Effect Level (LO(A)EL) instead of the NO(A)EL**, the SCCS usually takes into consideration **an additional factor of 3** in the calculation of the MoS. This is decided upon on a case-by-case basis, taking into account the dose spacing in the performed repeated dose toxicity test, the shape and slope of the dose-response curve (and in some approaches the extent and severity of the effect seen at the LO(A)EL). Defaults typically suggested for this assessment factor range from 1–10 [EChA 2008b].

Finally, in case no 90 day study is available, a default assessment factor from subacute (28 days) to subchronic (90 days) toxicity of 3 can be considered [EChA 2008b].

c) The Systemic Exposure Dosage (SED)

Generally, the systemic availability of a cosmetic ingredient is estimated by taking into account the daily amount of finished cosmetic product applied, the concentration of the ingredient under study, the dermal absorption of that particular ingredient and a mean

human body weight value. As such, the amount of ingredient per kg body weight that would become available daily in the human circulatory system, is calculated.
However, in the majority of MoS calculations, this **dermal** exposure figure is compared to an **oral** NO(A)EL value, which corresponds to the amount that has been administered orally, though not necessarily to the actual systemic availability of the compound after oral administration.
The SCCS acknowledges the fact that in many **conventional calculations** of the MoS, the **oral bioavailability** of a substance is **assumed to be 100%** if oral absorption data are unavailable. However, it is considered **appropriate to assume that not more than 50% of an orally administered dose is systemically available**. The value of 50% is an arbitrary choice that recognises that the gastrointestinal tract is designed to favour the absorption of ingested substances into the body but that, in most cases, not all of the ingested material will be bioavailable. Thus, in the absence of data, the assumption is being made that effects seen following oral administration have been caused by a fraction of the administered dose and not the entire administered dose. If there is **evidence to suggest poor oral bioavailability**, for example the substance is a poorly soluble particulate, it may be more appropriate to assume that only **10%** of the administered dose is **systemically available** [IGHRC 2006]. Whenever oral absorption data are available, these should be included in the calculations [e.g. SCCP/0851/04].

In the case of **oral-to-inhalation extrapolation**, it was proposed that, in the absence of route-specific bioavailability information, a **default factor of 2** (i.e. the absorption percentage for the starting route is half that of the end route) **might be appropriate**. The inclusion of this factor 2 means for example that 50% (instead of 100%) absorption is assumed for oral absorption, and 100% for inhalation.
Any route-to-route extrapolation can be performed in a case-by-case manner based on expert judgment of scientific information, including available toxicokinetic information. It can, however, only be performed in the case of systemic toxicity. Not only the degree of absorption, but also metabolism should be considered.

An additional remark with regard to MoS calculations is whether such calculations are scientifically relevant for cosmetic ingredients which are not used on a daily basis, i.e. cosmetics with intermittent exposure. Comparing a monthly usage level with a NO(A)EL value obtained after daily administration of the substance, is a clear overestimation of the risk. This discussion is not restricted to cosmetic products, but to the risk assessment procedure of all dangerous substances in the EU.
The SCCS can accept on a case-by-case basis the comparison of a NO(A)EL resulting from a daily exposure study with the SED of that product, even if it is only applied e.g. once per month. Note that the repeated exposure resulting from a certain exposure scenario is to be expressed as the actual daily dose, bearing in mind that for consumers a 'day' may vary between 1 and 24 hours (depending on the scenario, e.g., type of consumer product). The actual daily dose is *independent* of the exposure frequency. This means that if, for a certain scenario, worker or consumer exposure is only for a number of days per year, the exposure value is the actual dose on the exposure days, and not the daily dose averaged out (and thus divided!) over the whole year [EChA 2008b]. This reasoning however, may be changed for example in the case of hair dyes and a MoS slightly below 100. One could consider a substance as being safe due to the occasional use and the built-in conservatism of assessment, but only after expert judgment.
Therefore, the SCCS will decide upon the relevance of MoS calculations on a case-by-case basis, taking into account the general toxicological profile of the substance under consideration, its toxicokinetic properties and its intended use.

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. In that case, the default value of involved skin surface area (SSA) needs to be known per product type (see Table 2, section 4-2) to estimate the systemic availability of the ingredient.

Calculations of the SED may also be based on the **percentage** dermally absorbed. The resulting numbers will then depend on amount of finished product applied on the skin (see Table 3, section 4-2 for default values per product type). In this case, the concentrations tested should also include the lowest concentration anticipated.

According to OECD 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 30-45 minutes (depending on the intended use).

Experience has shown that *in vitro* measurements using less than 2 mg/cm² are not technically feasible while the amounts of cosmetic products applied to skin usually do not exceed 1 mg/cm² under in use conditions. Thus the *in vitro* tests are performed with applied amounts exceeding the intended use conditions and if the resulting dermal absorption % of the test dose is used to calculate SED, they may result in an underestimation of systemic exposure.

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 [SCCP/0970/06].

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

With:
- SED (mg/kg bw/day) = Systemic Exposure Dosage
- DA_a (µg/cm²) = Dermal Absorption reported as amount/cm², resulting from an assay under in-use mimicking conditions[1]
- SSA (cm²) = Skin Surface Area expected to be treated with the finished cosmetic product (see section 4-2 for SSA values per product type)
- F (day^{-1}) = Frequency of application of the finished product
- 60 kg = default human body weight

[1] In case the *in vitro* dermal absorption assay was not performed under in-use conditions, an additional correction factor can be introduced.

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

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

The calculation of the SED will be as follows:

SED = **A** (mg/kg bw/day) x **C** (%)/100 x **DA_p** (%)/100

With:
- SED (mg/kg bw/day) = Systemic Exposure Dosage
- A (mg/kg bw/day) = Estimated daily exposure to a cosmetic product per kg body weight, based upon the amount applied and the frequency of application (for calculated relative daily exposure levels for different cosmetic product types, see Table 3, section 4-2).
- C (%) = Concentration of the ingredient under study in the finished cosmetic product on the application site
- DA_p (%) = Dermal Absorption expressed as a percentage of the test dose assumed to be applied in real-life conditions[1]

If the application mode is such that the number of applications differs from the standard range 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. This conservative value may also be used in cases where no or inadequate absorption data are available (see 3-4.4 for more details on default dermal absorption data).

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, whereas a higher factor of 3.2 is generally foreseen by the WHO for the variability in human kinetics (See 3-7.2).

[1] In case the *in vitro* dermal absorption assay was not performed under in-use conditions, an additional correction factor can be introduced.

It can thus be stated that the inter-individual 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 The Threshold of Toxicological Concern (TTC)

The use of the TTC approach for cosmetics and consumer products has recently been evaluated by the SCCS/SCHER/SCENHIR (final report expected in 2011).

The TTC concept is a risk assessment tool trying to identify exposure levels below which no toxicity is expected to occur. Currently, it has been used for food contact materials (only in the USA), food flavourings, genotoxic impurities in pharmaceuticals and for pesticide metabolites in ground water. The use of this approach has been suggested for a number of other application areas.

The TTC concept is based on the principle of establishing a generic human exposure threshold value for chemicals, below which there is a low probability of systemic adverse effects to human health. The concept is based on extrapolation of toxicity data from an available database to a chemical compound for which the chemical structure is known, but no or limited toxicity data is available. At present, a database containing carcinogenicity data from animal studies for 730 chemicals (Carcinogen Potency Database, CPDB) and one database containing 613 chemicals based on other toxicological endpoints (Munro database) are available. Both are based on systemic effects after oral exposure.

Application of the TTC approach in risk assessment requires a high level of confidence in 1) the quality and completeness of the databases and 2) the reliability of the exposure data for the intended use of the compound under study. Several chemical classes/endpoints have been identified for which the TTC approach is not considered to be applicable. In addition, many complex chemical structures are not adequately represented in the available databases. The influence of individual functional groups is unknown and any receptor mediated effects of the parent compound or its metabolite(s) are difficult to assess. Endpoints which are not included in the databases used for the extrapolation of a TTC need to be considered separately, e.g. local effects. When using the TTC approach, any available information on the compound should be considered. This should also include structure activity relationships and read-across analyses.

The TTC approach in itself is scientifically acceptable. In principle, it is applicable to any chemical be it an intentionally added ingredient or a chemical present in a particular product as inadvertent contaminant or impurity. In relation to cosmetic ingredients, however, the current databases require further development and validation. Therefore, the TTC approach is at present not generally applicable for intentionally added ingredients present in cosmetic products. In the case of chemicals from a structural class well represented in the TTC database, an application of the TTC approach for cosmetic ingredients can be considered on a case-by-case basis.

3-7.6 Assessment of carcinogens

Non-genotoxic carcinogens

The distinction between carcinogens likely to cause tumours by interaction with the genetic material (genotoxic) and carcinogens causing tumours by other mechanisms not involving genotoxicity (non-genotoxic) is a major determinant for the selection of risk assessment methodologies. Genotoxic agents are considered not to have a threshold. Instead they are expected to induce increases in DNA damage linearly related to the administered dose. It is also theoretically assumed that even a single molecule of a genotoxic carcinogen may cause a mutation and thus result in an increased cancer incidence, although the increase in risk may be infinitesimally small. Non-genotoxic carcinogens are assumed to have a threshold.

In cases of non-genotoxic carcinogens where a threshold for induction of tumours has been identified, the risk assessment is performed as for other toxicological endpoints with a threshold, by calculation of a MoS.
The decision on a threshold and a non-threshold mode of action of a carcinogenic agent may not always be easy to make. In some cases the possible involvement of a genotoxic mechanism may be difficult to determine, in other cases, although a biological threshold may be postulated, the data do not allow its identification. If a threshold is not clear, the assumption of a non-threshold mode of action would be the prudent choice [EChA 2008b]. Thus, risk assessment for such carcinogens should be performed as for non-threshold carcinogens.

The linear extrapolation approach (T_{25} method)
Three methods for quantitative risk characterisation have been used by regulatory authorities in Europe and USA. The "Linearised Multistage Model" was previously extensively used by the US EPA [1986]. The "Lowest Effective Dose $(LED)_{10}$ method" has been used more recently by the US EPA [1996] and the "T_{25} method" [Sanner et al. 2001] is the default method for quantitative risk assessment of carcinogens in the EU [EChA 2008b]. The results obtained with the three methods are in most cases quite similar. It should be noted that, in cases where high quality epidemiology and animal carcinogenicity studies are available, a good agreement was found between hazard characterisation based on epidemiology and hazard characterisation based on animal studies using the T_{25} method [Sanner and Dybing 2005a].

Determination of the lifetime cancer risk is carried out in different steps. After having decided what animal data set to be used and type of tumour to consider, the dose descriptor T_{25} is determined. T_{25} is defined as the chronic dosage rate that will give 25% of the animals' 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 detail 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 systemic exposure dosage (SED), the lifetime cancer risk is calculated by linear extrapolation by use of the following formula:

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

Subsequently, a statement is generated describing whether the actual risk may be higher or lower than the risk calculated for a specific scenario. The procedure is reported in detail by Sanner et al. [2001] and [EChA 2008b].

The decision on the threshold for concern with regard to the calculated lifetime cancer risk, is a political issue. Some countries and international organisations have considered that a lifetime cancer risk in the general population of less than 10^{-5} to 10^{-6} is of little or no concern. This can be used as a guiding principle.

The Margin of Exposure (MoE) approach
The European Food Safety Authority (EFSA) recommends application of the concept of MoE for assessing the risk of genotoxic and carcinogenic substances [EFSA 2005]. The MoE represents the ratio between the dose descriptor for tumour formation in animals and the daily systemic human dose (SED). Depending on the quality of the animal carcinogenicity data and the number of dose levels used in these studies, the dose-descriptors T_{25} or the $BMDL_{10}$ are used as dose descriptors.

EFSA [2005] concluded "that a MoE of 10,000 and above, based on a $BMDL_{10}$ or 25,000 and above based on T_{25} from an animal study, would be a value that would indicate a low concern from a public health point of view and that might be considered a low priority for risk management actions". The MoE of 10,000 is based on the assumption that for genotoxic carcinogens the products of four 10-fold factors are needed. The two first allows for possible inter-species and intra-species differences, the third takes into account the additional uncertainties related to the carcinogenic process and the fourth the use of $BMDL_{10}$ instead of a NO(A)EL). According to quantitative risk characterisation based on the T_{25} method, this would correspond to a lifetime cancer risk of about $7x10^{-5}$ in the case of a mouse experiment and about $3.5x10^{-5}$ if based on a rat experiment.

Genotoxic substances assumed to be carcinogens

At present no quantitative or semi-quantitative method has been accepted for regulation of genotoxic/mutagenic agents. The possible carcinogenic effects of genotoxic agents are generally considered to be more critical than germ cell mutagenesis with regard to exposure to chemical mutagens. Hence, the finding of a linear relationship between the LED for *in vivo* genotoxicity and the carcinogen dose descriptor T_{25}, is of importance [Sanner and Dybing, 2005b]. It was found for the 34 carcinogens studied which covered a potency range of 10,000, that the median of the ratio LED/T_{25} was equal to 1.05 and that for 90% of the substances the numerical value of LED was similar to the numerical value of T_{25} within a factor of less than 5–10. The results suggest that if further evaluated, LED for *in vivo* genotoxicity probably could be used in a semi-quantitative method for risk assessment of mutagens without a long-term study.
For genotoxic carcinogens the Scientific Committees of DG SANCO [SCCP, SCHER, SCENIHR, 2009] recommend in agreement with the European Chemical Agency [EChA 2008b] two default methodologies for deriving an exposure level of little or no concern. The linear extrapolation approach or T_{25} method, as described above, results in a lifetime cancer risk considered being of low concern while the Margin of Exposure approach supports priority setting for risk management actions (a MoE of 10,000 would be considered as a low concern for genotoxic substances).

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 tumour 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.

Intermittent exposure to genotoxic carcinogens: The human dose is determined on the basis of a relevant scenario or measurements and the lifetime cancer risk is subsequently calculated. If the exposure is less than lifetime a correction factor should be applied to the calculated chronic exposure dose.
For workers with an exposure time of 8 hours per day, 5 days per week, 48 weeks per year for 40 years the default correction factor is 2.8 (7/5 x 52/48 x 75/40) for oral studies, Analogously, for 4-aminobiphenyl (4-ABP) as a contaminant in hair dyes under the assumption that a the permanent hair dye is used once per month the average daily dose was corrected according to the frequency of exposure by dividing the estimated exposure dose by 30 [SCCNFP/0797/04, SCHER/SCCP/SCENIHR 2009, EChA 2008b].

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 2005, the European Food Safety Authority requested its Scientific Committee to issue an opinion on a harmonised approach to for the risk assessment of substances with both genotoxic and carcinogenic properties.

In this report, not only the T_{25} method is described, but also the so-called "TD_{50}" and "Benchmark Dose" approaches:

- The TD_{50} is defined as the chronic dosage rate (in mg/kg bw per day) which, for a given target site(s), would cause tumours in half of the animals within some standard experimental time – the "standard lifespan" for the species. A TD_{50} can be calculated either for a particular category of neoplastic lesion (e.g. malignant tumours only, liver tumours only) or for all tumours.
- The selection of NOAEL or LOAEL is usually based on the dose levels used in the most relevant toxicity study, without considering the shape of the dose response curve. The value obtained depends on the selection of doses (spacing) and a small group of animals used tend to result in higher NOAELs. Therefore, the NOAEL/LOAEL may not reflect the true threshold for the adverse effect. Alternatively, the Bench Mark Dose (BMD) may also be used as the starting point for the derivation of the Margin of Safety. The calculation of a BMD is a statistical approach for the determination of the threshold and relies on the dose response curve. Mathematical curve fitting techniques can be used for determining a negligible adverse effect. By using a suitable model (probit, logit, Weibull etc.), a benchmark response including the confidence level is calculated, e.g. 5% response at 95% lower confidence level. In this way a $BMDL_{05}$ is derived where the response is likely to be smaller than 5%.

The critical effect would be selected as the endpoint resulting in the lowest BMDL. The BMD can be used in parallel to derivation of a NOAEL or as an alternative when there is no reliable NOAEL [EFSA 2008, EChA 2008b].

Viewing the fact that the BMD is typically accomplished through dose-response modelling considering all available information on the dose response curve whereas the T_{25} calculation is based upon one data point on the dose-response curve, the EFSA Scientific Committee expressed its preference for the BMD approach. However, in case the available data are found to be inadequate for the estimation of a benchmark dose lower confidence limit, it was advised to use the T_{25}, representing the (corrected) dose corresponding to a 25% tumour incidence [EFSA 2005].
Basically, the EFSA approach results into a margin of exposure (MoE), in analogy with the margin of safety (MoS) and is not quantifying human risk.

To date, the SCCS / SCC(NF)P has mainly used the T_{25} approach in order to determine the lifetime cancer risk of cosmetic ingredients and contaminants.

3-8 THE SPECIFIC ASSESSMENT OF HAIR DYES AND HAIR DYE COMPONENTS

3-8.1 Hazard and risk assessment of hair dyes in general

With regard to the assessment of hair dyes in general, different approaches are supported for either (i) temporary, (ii) semi-permanent or (iii) permanent hair dyes. It was the opinion of the SCCNFP that priority should be given to the evaluation and regulation of oxidative (permanent) hair dyes [SCCNFP/0959/05]. Since these hair dyes typically consist of a two component system, leading to a chemical reaction after mixing, the safety assessment should take into account that the consumer will potentially be exposed to precursor(s), coupler(s), intermediate(s) and end products [SCCNFP/0566/02, SCCNFP/0808/04, SCCP/0941/05, SCCP/1004/06]. Finally, the SCCP experts pointed out that the aspect of allergenicity of the different compounds has not been addressed yet [SCCP/0941/05, SCCP/1004/06]. Colipa recommends a self test of consumers "Perform a skin allergy test 48 h before each product use" and several companies label their hair dye products with this kind of safety instruction. With regard to consumer self testing SCCP pointed out that this may lead to misleading results and to skin sensitisation and that the use of hair dye products on the skin and for in vivo diagnostic purposed is not covered by the current Cosmetics directive [SCCP/1104/07].
The major concern in the safety assessment of hair dye formulations, however, is the putative link between their use and the development of cancer. Several SCC(NF)P opinions have stated the conclusion that the potential risk of developing cancer due to the use of certain hair dyes gives rise to concern [SCCNFP/0484/01, SCCNFP/0797/04, SCCP/0930/05] and that the assessment should focus on leukaemia and bladder cancer, since no evidence was found linking personal use of hair dyes to a cancer risk at other sites [SCCP/0930/05].

3-8.2 Step-wise regulation of hair dyes

In April 2003 the Commission together with the Member States agreed on a step-wise strategy[1] to regulate all hair dyes listed as ingredients in cosmetic products. The main element of the strategy was a tiered, modular approach, requiring industry to submit by certain deadlines safety dossiers for hair dye components and possible mixtures. This strategy was supported by the SCCNFP through its "Opinion on hair dyes without file submitted", in which the experts clearly expressed the demand for a safety dossier for all hair dyes, irrespective whether they had already been taken up in one of the annexes of Directive 76/768/EEC [SCCNFP/0807/04]. The SCCS differentiates between temporary, semi-permanent and permanent hair dyes [SCCP/0959/05].

[1] Available through http://europa.eu.int/comm/enterprise/cosmetics/html/cosm_ongoing_init.htm.

To ensure the safety of hair dye products, the Commission decided to ban all permanent and non-permanent hair dyes for which industry did not submit any safety files and those for which the SCCP had given a negative opinion [IP/06/1047]. Over time, 106 hair dye substances were taken up in Annex II to Directive 76/768/EEC [2006/65/EC, 2007/1/EC, 2007/54/EC]. According to the safety files received, 48 oxidative hair dyes are now commercially used in the EU, of which 16 are precursors and 32 are couplers.

3-8.3 MoS calculations for hair dye formulations

1. Dermal absorption and SED-related default values for hair dyes

In dermal absorption studies with hair dye formulations and ingredients, usually an **amount of 20 mg/cm² is applied for 30-45 minutes** (depending on the intended use). Regularly, the dermal absorption value is expressed as amount/cm² and a default surface of the scalp of 700 cm² has been used in order to maintain consistency among the opinions [e.g. SCCNFP/0657/03 and SCCNFP/0669/03]. The SCCS Working Group on Hair Dyes decided to change to the more commonly used **scalp surface area value of 580 cm²** in its evaluations.

2. Intermittent exposure and MoS calculations

It is acknowledged that the calculation of a MoS for hair dyes is scientifically debatable, since the dyes are not intended to be applied on a daily basis. However, it was noted that the repeated exposure resulting from a certain exposure scenario is to be expressed as the actual daily dose, bearing in mind that for consumers a day may vary between 1 and 24 hours (depending on the scenario, e.g. type of consumer product). The actual daily dose is *independent* of the exposure frequency. This means that if, for a certain scenario, **worker or consumer exposure is only for a number of days per year, the exposure value is the actual dose on the exposure days**, and not the daily dose averaged out (and thus divided!) over the whole year [EChA 2008b].

When assessing the risk of a genotoxic carcinogen in hair dye formulations, e.g. a hair dye contaminant, human systemic exposure may be adjusted according to the frequency to mean exposure per day assuming one hair colouring event every 28 days.

3-8.4 Mutagenicity / genotoxicity testing of hair dye substances and reaction products

Viewing the putative link between the use of hair dyes and cancer development, the mutagenic potential of the different hair dye components has received a great deal of attention [SCCNFP/0720/03, SCCNFP/0808/04, SCCP/0941/05].
The testing strategy for testing hair dye cosmetic ingredients for their potential mutagenicity was firstly issued in 2002 [SCCNFP/0566/02] and has been updated twice [SCCNFP/0720/03, SCCP/0971/06]. SCCP/0971/06 provided a stepwise *in vitro* strategy for hazard identification with regard to the mutagenic potential of hair dyes, so that sufficient *in vitro* data may be obtained.

To date, in the case of hair dyes there is no reason to deviate from the general strategy derived for cosmetic ingredients. More specifically, the recommended base set of *in vitro* mutagenicity assays for oxidative hair dye substances consists of 3 tests (see 3-4.6). Discussions are ongoing to see whether this can be reduced to 2 tests.

Meanwhile, the SCCS focused on the overall consumer health risk caused by products and intermediates of oxidative hair dyes formed during hair dyeing processes (including their potential mutagenic/genotoxic/carcinogenic properties). The following conclusions were drawn [SCCS/1311/10]:

- The use of oxidative hair dye formulations results in consumer exposure to precursors and couplers as well as to their reaction products. Exposure to reaction products is

considerably lower compared to that from precursors and coupler. No exposure to intermediates was noted.

- The percutaneous absorption rates in the *in vitro* skin penetration studies of the 14 representative reaction products evaluated ranged from 3.27 to 717.79 ng/cm^2 (mean + 1SD). This corresponds to 1.9 to 416 µg absorbed dose (i.e. dose potentially bioavailable) per hair dye application (i.e. 0.03 to 6.9 µg/kg bw).
- In the risk assessment of reaction products general toxicity is not considered a concern due to the low and intermittent exposure (on average once per month).
- As no data has been made available for this endpoint, sensitization risk is not addressed.
- For genotoxicity, a common result for both precursors/couplers and the reaction product is the positive outcome in one or more *in vitro* tests which was not confirmed *in vivo*. It can be deduced that it is not possible to predict the specific outcome of the tests of the reaction product on the basis of the results of the respective precursors/couplers. A final conclusion on the possible genotoxic hazard can be drawn only on the basis of testing.
- The use of (Q)SAR in the case of reaction products was of limited value since the arylamine structure, a structural element of many hair dye precursors and reaction products, is automatically identified as an alert. For the assessment of arylamine-containing complex molecules it is desirable to use or to develop in the future SAR for *in vivo* genotoxicity which satisfies the OECD principles and has a known applicability domain.
- With regard to the carcinogenicity of oxidative hair dye formulations in humans, no clear-cut conclusion can be drawn from the studies. A definite answer to the question whether a causal relationship exists between personal hair dye use and cancer cannot be expected by epidemiology alone. From the evaluation of the available studies it can be deduced that for current users of hair dyes marketed in the EU no clear indications for an excess of cancer risk have been demonstrated. This judgement is in line with a recent evaluation of IARC: The Working Group considered the epidemiological evidence inadequate, and concluded that personal use of hair colorants is ”not classifiable as to its carcinogenicity in humans” (Group 3) [IARC 2010].
- It is common practice that oxidative hair dye formulations contain more than one precursor and coupler. Thus, the use of oxidative hair dyes may result in exposure to several reaction products simultaneously. This combined exposure has not been considered.

Based on the data yet available, the **SCCS raises no major concern regarding genotoxicity and carcinogenicity of hair dyes and their reaction products currently used in the EU**. However, at present, the database on genotoxicity of reaction products underpinning this conclusion is small and therefore some degree of uncertainty remains. Enlargement of the database with information on additional reaction products would strengthen the above conclusions. At present, confirmation of safety regarding genotoxicity and carcinogenicity could only be achieved by the use of *in vivo* studies, which, however, are no longer permitted according to EU legislation. In the future, modern methodologies (e.g. skin models, -omics, SAR) may allow the assessment of safety without animal experimentation.

4. SAFETY EVALUATION OF FINISHED COSMETIC PRODUCTS

4-1 INTRODUCTION

In accordance with the 6th [93/35/EEC] and 7th [2003/15/EC] Amendment to Council Directive 76/768/EEC, and with the upcoming requirements of the recast [2009/1223/EC], a product information file (PIF) 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 PIF 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.

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. However, some practical guidance is provided here. It should not be used as a checklist but rather as an approach to be adapted on a case-by-case basis when evaluating the safety of a finished cosmetic product.

4-2 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 the way 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),
- the ingredients of permanent hair dyes undergo oxidative reactions (e.g. with hydrogen peroxide) on the hair, precursors(s), coupler(s), intermediate(s) 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,
- 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 should also be considered (e.g. inhalation of spray products, ingestion of lip products, etc.).
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].

The following calculations take into account the **dermal** exposure to cosmetic products. Dependent on 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 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, resulting from an assay under in-use mimicking conditions[1]
- SSA (cm²) = Skin Surface Area expected to be treated with the finished cosmetic product (see section 4-2 for SSA values per product type)
- 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 2 are extracted from a Dutch study on cosmetic exposure assessment performed by the RIVM (RijksInstituut voor Volksgezondheid & Milieu) [Bremmer

[1] In case the *in vitro* dermal absorption assay was not performed under in-use conditions, an additional correction factor can be introduced.

et al. 2005] and indicate exposed skin surface areas per cosmetic product type[32]. The last column of the same table reflects the presumed **frequency of application (F)** of the finished product.

Table 2: Mean exposed skin surface area per product type [Bremmer et al. 2005] and frequency of application per product type

Product type	Skin surface area involved (RIVM)		Frequency of application*
	Surface area (cm²)	Parameters (if specified)	
Bathing, showering			
Shower gel	17500	total body area	*1.43/day*
Hand wash soap	860	area hands	10/day[33]
Bath oil, salts, etc.	16340	area body - area head	1/day
Hair care			
Shampoo	1440	area hands + 1/2 area head	1/day
Hair conditioner	1440	area hands + 1/2 area head	0.28/day
Hair styling products	1010	1/2 area hands + 1/2 area head	*1.14/day*
Semi-permanent hair dyes (and lotions)	580	1/2 area head	1/week (20 min.)
Oxidative/permanent hair dyes	580	1/2 area head	1/month (30 min.)
Skin care			
Body lotion	15670	area body - area head female	*2.28/day*
Face cream	565	1/2 area head female	*2.14/day*
Hand cream	860	area hands	2/day
Make-up			
Liquid foundation	565	1/2 area head female	1/day
Make-up remover	565	1/2 area head female	1/day
Eye shadow	24		2/day
Mascara	1.6		2/day
Eyeliner	3.2		2/day
Lipstick, lip salve	4.8[34]		*2/day*
Deodorant			
Deodorant aerosol	200	both axillae	*2/day*

* Frequency figures in *italics* correspond to the 90th percentile values of the 2005/2009 Colipa studies (see next paragraphs for details on Colipa studies)

[32] Besides these European values, it should be noted that the US EPA also published default values for skin surface areas of relevant parts of the human body [US EPA 1997].

[33] Danish Ministry of the Environment, Environmental Protection Agency: Survey of liquid hand soaps, including health and environmental assessments.

[34] Ferrario et al. 2000.

Product type	Skin surface area involved (RIVM)		Frequency of application*
	Surface area (cm^2)	Parameters (if specified)	
spray[1] and non-spray[2]			
Fragrances			
Eau de toilette spray	200		1/day
Perfume spray	100		1/day
Men's cosmetics			
Shaving cream	305	1/4 area head male	1/day
Aftershave	305	1/4 area head male	1/day
Sun care cosmetics			
Sunscreen lotion / cream	17500	total body area	2/day

* Frequency figures in *italics* correspond to the 90th percentile values of the 2005/2009 Colipa studies (see next paragraphs for details on Colipa studies)

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

The calculation of the SED will be as follows:

$$SED = \mathbf{A}\ (mg/kg\ bw/day) \times \mathbf{C}\ (\%)/100 \times \mathbf{DA_p}\ (\%)/100$$

With:
- SED (mg/kg bw/day) = Systemic Exposure Dosage
- A (mg/kg bw/day) = Estimated daily exposure to a cosmetic product per kg body weight, based upon the amount applied and the frequency of application: see the calculated relative daily exposure levels for different cosmetic product types in Table 3
- 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 of the test dose assumed to be applied in real-life conditions[3]

In this case it is key to know the **daily amount of formulation applied per kg body weight (A)** under intended in use conditions.

For many years, the Notes of Guidance have displayed the same set of cosmetic exposure data provided by Colipa. Upon repeated request of the SCC(NF)P / SCCS, more recent and robust data were provided for 6 product types (body lotion, deodorant, facial moisturiser, shampoo, lipstick and toothpaste) in 2005 [Hall et al. 2007, McNamara et al. 2007] and for 5 additional product types (mouthwash, shower gel, liquid foundation, hand cream and hair styling products) in 2009 [Hall et al. 2011]. The results are based upon a large-scale study among consumers in different European Member States reporting on their personal use of cosmetic products. In order to provide a pertinent prediction for the European population, the exposure data were generated using probabilistic analysis [Hall et al. 2007, 2011].

1 2010 Colipa study, Steiling et al. (publication in preparation); results presented to the SCCS.

2 Cowan-Ellsberry et al. 2008.

3 In case the *in vitro* dermal absorption assay was not performed under in-use conditions, an additional correction factor can be introduced.

As such, the new figures for the daily amounts of cosmetic products can be incorporated in the current Notes of Guidance. In the Colipa studies, it was shown that for many product types there is an **inverse** correlation between the frequency of product use and the quantity used per application. Since the amount of product applied declines with frequency of use, it is no longer appropriate to calculate daily exposure by simple multiplication of the maximum frequency per day value by the maximum quantity per application value as was done before in the previous versions of the Notes of Guidance.

Therefore, Table 3 displays the daily amount applied and the retention factor[1] to come to the final daily dermal exposure to the finished product. For the product types included in the recent Colipa studies this daily amount applied is a 90th percentile taken from the distribution of measured values. For the data already present in previous versions of the Notes of Guidance and for which no new empirical data are available, the calculation of the maximum frequency per day multiplied by the maximally applied amount still stands.
In case the safety assessor of a finished product wishes to know the average use frequency related to the obtained data, reference is made to Table 2, which displays skin surface area involved, and also the assumed frequency of use.

A new feature in the calculation and Table 3 is the fact that the body weight is already incorporated in the daily amount of product applied. This accounts for the Colipa test setting in which distributions of amounts of products used per day were probabilistically divided by distributions of body weights reported for the EU countries by ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals). The values given in Table 3 correspond to the 90th percentile[2]. In case of product types for which such data was not available, the 'old' application value (as given in the previous Notes of Guidance) was divided by the mean human body weight of 60kg.

The SCCS emphasises that it is not the intention to provide exposure data for **all** cosmetic product types. Only the most common products are displayed. For all other end products, it is up to the individual companies and/or the qualified safety assessors to make a case-by-case assessment of the daily exposure level and/or the frequency of application.

1 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]

2 The body weights used were thus not the body weights of the volunteers in the study but elsewhere reported population body weights in the studied countries.

Table 3: Estimated daily exposure levels for different cosmetic product types according to Colipa data [SCCNFP/0321/02; Hall et al. 2007, 2011].

Product type	Estimated daily amount applied	Relative amount applied (mg/kg bw/day)	Retention factor [1]	Calculated daily exposure (g/day)	Calculated relative daily exposure (mg/kg bw/day)
Bathing, showering					
Shower gel	18.67 g	279.20	0.01	0.19	2.79
Hand wash soap [2]	20.00 g	-	0.01	0.20 [3]	3.33
Hair care					
Shampoo	10.46 g	150.49	0.01	0.11	1.51
Hair conditioner [2]	3.92 g	-	0.01	0.04	0.60
Hair styling products	4.00 g	57.40	0.1	0.40	5.74
Semi-permanent hair dyes (and lotions) [2]	35 ml (per application)	-	0.1	Not calculated	-
Oxidative/permanent hair dyes [2]	100 ml (per application)	-	0.1	Not calculated [4]	-
Skin care					
Body lotion	7.82 g	123.20	1.0	7.82	123.20
Face cream	1.54 g	24.14	1.0	1.54	24.14
Hand cream	2.16 g	32.70	1.0	2.16	32.70
Make-up					
Liquid foundation	0.51 g	7.90	1.0	0.51	7.90
Make-up remover [2]	5.00 g	-	0.1	0.50	8.33
Eye shadow [2]	0.02 g	-	1.0	0.02	0.33
Mascara [2]	0.025 g	-	1.0	0.025	0.42
Eyeliner [2]	0.005 g	-	1.0	0.005	0.08
Lipstick, lip salve	0.057 g	0.90	1.0	0.057	0.90
Deodorant					
Deodorant non-spray	1.50 g	22.08	1.0	1.50	22.08
Deodorant aerosol spray (ethanol-based) [5]	1.43 g	20.63	1.0	1.43	20.63
Deodorant spray (not ethanol-based)	0.69 g	10.00	1.0	0.69	10.00
Oral hygiene					
Toothpaste (adult)	2.75 g	43.29	0.05	0.138	2.16
Mouthwash	21.62 g	325.40	0.10	2.16	32.54

1 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]

2 Product types not covered by the Colipa studies: existing daily application amounts are divided by the mean human body weight of 60 kg.

3 Danish Ministry of the Environment, Environmental Protection Agency: Survey of liquid hand soaps, including health and environmental assessments.

4 Daily exposure value not calculated due to the low frequency of exposure (see also 3-8.3.1).

5 Steiling et al. (publication in preparation); results presented to the SCCS.
'Ethanol-based' are products containing ethanol as principal ingredient.

For a **sunscreen lotion**, an application of 18.0 g/day is assumed to be a realistic value [SCCNFP/0321/02].

For some cosmetic ingredients, individual product type exposure values as mentioned in Table 3 might not reflect the overall exposure to these compounds, since there is a clear possibility that they 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. This aggregate exposure is currently assessed on a case-by-case basis.

In the specific case of preservatives, the SCCNFP proposed to calculate a **global daily exposure value** for all cosmetic products that one person may daily apply on the skin [SCCNFP/0321/00]. Taking into account the latest exposure values and considering the worst-case scenario in which the consumer would use a set of cosmetic products containing the same preservative, an aggregate value of **17.4 g/day** or **269 mg/kg bw/day** will have to be used in the calculation of the MoS (see Table 4).
Sunscreens are not taken up in this list since they are only used in limited time periods of the year.

Table 4: Calculation of aggregate exposure through cosmetic use for preservatives.

Type of exposure	Product	g/day	mg/kg bw/day
Rinse-off skin & hair cleansing products	Shower gel	0.19	2.79
	Hand wash soap	0.20	3.33
	Shampoo	0.11	1.51
	Hair conditioner	0.04	0.67
Leave-on skin & hair care products	Body lotion	7.82	123.20
	Face cream	1.54	24.14
	Hand cream	2.16	32.70
	Deo non-spray	1.50	22.08
	Hair styling	0.40	5.74
Make-up products	Liquid foundation	0.51	7.90
	Make-up remover	0.50	8.33
	Eye make-up	0.02	0.33
	Mascara	0.025	0.42
	Lipstick	0.06	0.90
	Eyeliner	0.005	0.08
Oral care cosmetics	Toothpaste	0.14	2.16
	Mouthwash	2.16	32.54
TOTAL		**± 17.4**	**269**

Although the dermal route is the most common one for cosmetic products, the consumer may also be exposed to cosmetic ingredients through inhalation (e.g. through spray applications). However, no corresponding exposure values are taken up in Tables 3 and 4 and the inhalation risk is currently assessed on a case-by-case basis. An example is the recent SCCS opinion on Dihydroxyacetone (DHA), a self-tanning agent used in spraying booths. For each type of booth, the DHA concentration was monitored in the air and the SCCS based its exposure assessment upon default breathing volumes, measured air concentrations, particle sizes and exposure duration under different settings [SCCS/1347/10].
The SCCS envisages including in the next version of the Notes of Guidance the most commonly encountered exposure scenarios for sprays and aerosols.

4-3 GUIDELINES FOR THE SAFETY EVALUATION OF FINISHED COSMETIC PRODUCT S

4-3.1 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].

4-3.2 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 (taking existing EU legislations into consideration):

- *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 material 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, …).

4-3.3 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 mixture (emulsion o/w or w/o, suspension, lotion, powder, aerosol, …);
- organoleptic properties (colour, odour, whenever relevant);
- pH (at ..°C) for aqueous mixtures;
- 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.

4-3.4 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 SCCS.

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

4-4.1 Preamble

Skin and mucous membranes are protected from microbial attack by a natural mechanical barrier and various defence mechanisms. However, these 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.

4-4.2 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 when tested 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 when tested 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.

4-4.3 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.

4-4.4 Good Manufacturing Practice

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.
Compliance should be checked with the currently available CEN standards (available through http://www.cenorm.be/cenorm/index.htm) and/or ISO standards (available through http://www.iso.org/iso/en/ISOOnline.frontpage).

5. REFERENCE LIST

67/548/EEC - Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.
Official Journal P 196, 16/08/1967 p.1.

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.

78/45/EEC - Commission Decision 78/45/EEC of 19 December 1977 establishing a Scientific Committee on Cosmetology.
Official journal L 13, 17/01/1978 p.24.

86/609/EEC - Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes.
Official Journal L 358, 18/12/1986 p.1.

87/18/EEC - Council Directive 87/18/EEC of 18 December 1986 on the harmonisation of laws, regulations and administrative provisions relating to the application of the principles of good laboratory practice and the verification of their applications for tests on chemical substances.
Official Journal L 15, 17/01/1987 p.29.

89/48/EEC - Council Directive 89/48/EEC of 21 December 1988 on a general system for the recognition of higher-education diplomas awarded on completion of professional education and training of at least three years' duration.
Official Journal L 19, 24/01/1989 p.16.

92/32/EEC - Council Directive 92/32/EEC of 30 April 1992 amending for the seventh time Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.
Official Journal L 154, 05/06/1992 p.1.

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.

93/67/EEC - Commission Directive 93/67/EEC of 20 July 1993 laying down the principles for assessment of risks to man and the environment of substances notified in accordance with Council Directive 67/548/EEC.
Official Journal L 227, 08/09/1993 p.9.

94/60/EC - European Parliament and Council Directive 94/60/EC of 20 December 1994 amending for the 14th time Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations.
Official Journal L 365, 31/12/1994 p.1.

97/1/EC - 20th Commission Directive 97/1/EC of 10 January 1997 adapting to technical progress Annexes II, III, VI and VII of Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products.
Official Journal L 16, 18/01/1997 p.85.

97/56/EC - Directive 97/56/EC of the European Parliament and of the Council of 20 October 1997 amending for the 16th time Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations.
Official Journal L 333, 04/12/1997 p.1.

97/579/EC - Commission Decision 97/579/EC of 23 July 1997 setting up Scientific Committees in the field of consumer health and food safety.
Official Journal L 237, 28/08/1997 p.18.

98/8/EC - Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market.
Official Journal L123, 24/04/1998 p.1.

1999/43/EC - Directive 1999/43/EC of the European Parliament and of the Council of 25 May 1999 amending for the 17th time Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the Member States relating to restrictions on the marketing and use of certain dangerous substances and preparations.
Official Journal L 166, 01/07/1999 p.87.

1999/45/EC - Directive 1999/45/EC of the European Parliament and of the Council of 31 May 1999 concerning the approximation of the laws, regulations and administrative provisions of the Member States relating to the classification, packaging and labelling of dangerous preparations.
Official Journal L 200, 30/07/1999 p.1.

2001/59/EC - Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.
Official Journal L 225, 21/08/2001 p.1.

2002/34/EC - Twenty-sixth Commission Directive 2002/34/EC of 15 April 2002 adapting to technical progress Annexes II, III and VII to Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to cosmetic products.
Official Journal L 102, 18/04/2002 p.19.

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.

2004/93/EC - Commission Directive 2004/93/EC of 21 September 2004 amending Council Directive 76/768/EEC for the purpose of adapting its Annexes II and III to technical progress
Official Journal L 300, 25/09/2004 p.13.

2004/210/EC - Commission Decision of 3 March 2004 setting up Scientific Committees in the field of consumer safety, public health and the environment
Official Journal L66, 04/03/2004 p.45.

2005/42/EC - Commission Directive 2005/42/EC of 20 June 2005 amending Council Directive 76/768/EEC, concerning cosmetic products, for the purposes of adapting Annexes II, IV and VI thereto to technical progress
Official Journal L 158, 21/06/2005 p.17.

2005/52/EC - Commission Directive 2005/52/EC of 9 September 2005 amending Council Directive 76/768/EEC, concerning cosmetic products, for the purposes of adapting Annex III thereto to technical progress
Official Journal L 234, 10/09/2005 p.9.

2005/80/EC - Commission Directive 2005/80/EC of 21 November 2005 amending Council Directive 76/768/EEC, concerning cosmetic products, for the purposes of adapting Annexes II and III thereto to technical progress
Official Journal L 303, 22/11/2005 p.32.

2006/65/EC - Commission Directive 2006/65/EC of 16 December 2008 amending Council Directives 76/768/EEC, 88/378/EEC, 1999/13/EC and Directives 2000/53/EC, 2002/96/EC and 2004/42/EC of the European Parliament and of the Council in order to adapt them to Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures
Official Journal L 198, 20/07/2006 p.11.

2006/78/EC - Commission Directive 2006/78/EC of 29 September 2006 amending Council Directive 76/768/EEC, concerning cosmetic products, for the purposes of adapting Annex II thereto to technical progress.
Official Journal L 271, 30/09/2006 p.56.

2006/257/EC - Commission Decision 2006/257/EC of 9 February 2006 amending Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products
Official Journal L 97, 05/04/2006 p.1.

2009/1907/EC - Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.
Official Journal L 396, 30/12/2006, p.1. Corrigendum in Official Journal L 136, 29/05/2007, p.3.

2007/1/EC - Commission Directive 2007/1/EC of 29 January 2007 amending Council Directive 76/768/EEC, concerning cosmetic products, for the purposes of adapting Annex II thereof to technical progress
Official Journal L 25, 01/02/2007 p.9.

2007/54/EC - Commission Directive 2007/54/EC of 29 August 2007 amending Council Directive 76/768/EEC, concerning cosmetic products, for the purpose of adapting Annexes II and III thereto to technical progress
Official Journal L 226, 30/08/2007 p.21.

2008/440/EC - Commission Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH)
Official Journal L 142, 31/05/2008 p.1.

2008/1272/EC - Regulation (EC) No 1272/2008 of the European Parliament and the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.
Official Journal L 353, 31/12/2008 p.1.

2009/1107/EC - Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC
Official Journal L 309, 24/11/2009 p.1.

2009/1223/EC - Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (recast)
Official Journal L 342, 22/12/2009 p.59.

Averbeck D., Moustacchi E.
Genetic effect of 3-Carbethoxypsoralen angelicin, psoralen and 8-methoxypsoralen plus 365 nm irradiation in Saccharomyces cerevisiae: induction of reversions, mitotic crossing-over, gene conversions and cytoplasmic "petite" mutations.
Mutation Research 68, 133-148 (1979).

Balls M. and Fentem J.H.
Progress toward the validation of alternative tests.
Alternatives To Laboratory Animals 25, 33-43 (1997).

Barile F.A. (Ed.)
Principles of Toxicology Testing.
CRC Press, Boca Raton, ISBN 0-8493-9025-7, 1-312 (2008).

Beck B.D., Calabrese E.J., Slayton T.M., Rudel R.
The use of toxicology in the regulatory process.
In: Principles and Methods of Toxicology. 5th Edition (A.W. Hayes, Ed.) CRC Press, Boca Raton, ISBN 0-8493-3778-X, 45-102 (2008).

Beronius A., Rudèn C., Hanberg A., Hakansson H.
Health risk assessment procedures for endocrine disrupting compounds within different regulatory frameworks in the European Union.
Regulatory Toxicology and Pharmacology 55, 111-22 (2009).

BfR (Bundesinstitut fur Risikobertung)
Strategies For Genotoxicity Testing Of Substances, November 2002.

Bremmer H.J., Prud'Homme de Lodder L.C.H., van Engelen J.G.M.
Cosmetics Fact Sheet to assess the risks for the consumer, Updated version for ConsExpo4.
RIVM Report 320104 001/2005 (2005).

Brendler-Schwaab S., Czich A., Epe B., Gocke E., Kaina B., Müller L., Pollet D., Utesch D.
Photochemical genotoxicity: principles and test methods - Report of a GUM task force.
Mutation Research 566(1), 65-91 (2004).

Chetelat A., Albertini S., Dresp I.H., Strobel R., Gocke E.
Photomutagenesis test development. Part I. 8-Methoxypsoralen, chloropromazine and sunscreen compounds in bacterial and yeast assays.
Mutation Research 292, 241-250 (1993a).

Chetelat A., Dresp J.H., Gocke E.
Photomutagenesis test development. Part II. 8-Methoxypsoralen, chlorpromazine and sunscreen compounds in chromosomal aberration assays using CHO cells.
Mutation Research 292, 251-258 (1993b).

Chetelat A., Albertini S., Gocke E.
The Photomutagenicity of fluoroquinolones in tests for gene mutation, chromosomal aberration, gene conversion and DNA breakage (Comet assay).
Mutagenesis 11(5), 497-504 (1996).

Colipa (The European Cosmetic Toiletry and Perfumery Association)
Scientific Committee on Cosmetology. Notes of guidance for the testing of cosmetic ingredients for their safety evaluation, 47-48, Brussels (1997).

Colipa (The European Cosmetic Toiletry and Perfumery Association)
European Consumer Exposure to Cosmetic Products - Part 1
Final Report, 17th September 2005.

Colipa (The European Cosmetics Association)
European Consumer Exposure to Cosmetic Products - Part 2
Final Report, 8th September 2009.

COM (UK Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment) Guidance on a strategy for Testing of Chemicals for mutagenicity. December 2000.

COM (UK Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment) Guidance on a strategy for Genotoxicity testing and mutagenic hazard assessment of chemical substances. Consultation document, issued November 2010, available through http://www.iacom.org.uk/publications/index.htm, consulted December 2010.

COM(97) 183 Final – Commission Communication on Consumer health and safety of 30 April 1997, available on the Internet through http://europa.eu.int/comm/food/fs/sc/comec1en.pdf [Aug 2003].

Cowan-Ellsberry C., McNamee P.M., Leazer T.
Axilla surface area for males and females: measured distribution.
Regulatory Toxicology and Pharmacology 52(1):46-52 (2008).

CSTEE (Scientific Committee on Toxicity, Ecotoxicity and the Environment)
Opinion of the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) on the BUAV-ECEAE report on "The way forward - action to end animal toxicity testing"
Doc. C7/VR/csteeop/anat/08014 D(04), European Commission (2004).

Damstra T., Barlow S., Bergman A., Kavlock R., Van Der Kraak G.
Global assessment of the state-of-the-science of endocrine disruptors.
IPCS (International Programme on Chemical Safety) Publication WHO/PCS/EDC/02.2 (2002).

Dean S.W., Lane M., Dunmore R.H., Ruddock S.P., Martin C.N., Kirkland D.J., Loprieno N.
Development of assays for the detection of photogenotoxicity of chemicals during exposure to UV light. Part 1. Assay development.
Mutagenesis 6, 335-341 (1991).

Dearfield K.L., Cimino M.C., McCarroll N.E., Mauer I., Valcovic L.R.
Genotoxicity risk assessment: a proposed classification strategy.
Mutation Research 521, 121-135 (2002).

Degen G.H. and Owens J.W. Xenoestrogens and xenoantiandrogens.
In: Greim H, Snyder R (Eds.): Toxicology and risk assessment: a comprehensive introduction, pp. 583-604, Hoboken, NJ: Wiley (2008).

Diembeck W., Beck H., Benech-Kieffer F., Courtellemont P., Dupuis J., Lovell W., Paye M. Spengler J., Steiling W.
Test Guidelines for *In Vitro* Assessment of Dermal Absorption and Percutaneous Penetration of Cosmetic Ingredients.
Food and Chemical Toxicology 37, 191-205 (1999).

DG ENV (Directorate-General Environment)
European Commission DG ENV - Groshart C.H. and Okkerman P.C. (authors)
Towards the establishment of a priority list of substances for further evaluation of their role in endocrine disruption – *preparation of a candidate list of substances as a basis for priority setting*.
Final Report M0355008/1786Q/10/11/00 (2000).

DG SANCO.
Sanco/222/2000: Guidance Document on Dermal Absorption. European Commission, Health and Consumer Protection Directorate-General, Doc. Sanco/222/2000 revision 7 of 19 March 2004.

Dybing E., Sanner T., Roelfzema H., Kroese D. and Tennant R.W.
T25: A simplified carcinogenic potency index: Description of the system and study of correlations between carcinogenic potency and species/site specificity and mutagenicity.
Pharmacology and Toxicology 80, 272-279 (1997).

EC - Guidelines for setting specific concentration limits for carcinogens in Annex I of directive 67/548/EEC. Inclusion of potency considerations.
Commission working group on the classification and labelling of dangerous substances.
Brussel (1999).
[Available through http://europa.eu.int/comm/environment/dansub/potency.pdf (09/2003)]

EC A.1 - Melting / freezing temperature
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.4

EC A.2 - Boiling temperature
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.14.

EC A.3 - Relative density
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.21.

EC A.4 - Vapour pressure
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.26.

EC A.6 - Water solubility
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.57.

EC A.8 - Partition coefficient
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.67.

EC A.9 - Flash-point
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.80.

EC A.15 - Auto-ignition temperature (liquids and gases)
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.104.

EC B.1 - Acute toxicity (oral)
Commission Directive 92/69/EEC of 31 July 1992 adapting to technical progress for the seventeenth 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.
Official Journal L 383A, 29/12/1992 p.110.

EC B.1 bis - Acute oral toxicity - Fixed Dose Procedure
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.145.

EC B.1 tris - Acute oral toxicity - Acute toxic class method
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.158.

EC B.3 - Acute toxicity (dermal)
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.178.

EC B.4 - Acute toxicity: dermal irritation / corrosion
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.182.

EC B.5 - Acute toxicity: eye irritation / corrosion
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.191.

EC B.6 - Skin sensitisation
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.202.

EC B.7 - Repeated dose (28 days) toxicity (oral)
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.210.

EC B.8 - Repeated dose (28 days) toxicity (inhalation)
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.216.

EC B.9 - Repeated dose (28 days) toxicity (dermal)
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.221.

EC B.10 - Mutagenicity - *in vitro* mammalian chromosome aberration test
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.225.

EC B.10 - Mutagenicity - *in vitro* mammalian chromosome aberration test
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.225.

EC B.13/14 - Mutagenicity - reverse mutation test using bacteria
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.248.

EC B.17 - Mutagenicity - *in vitro* mammalian cell gene mutation test
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.262.

EC B.18 - DNA damage and repair - unscheduled DNA synthesis - mammalian cells *in vitro*
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.271.

EC B.26 - Sub-chronic oral toxicity test: repeated dose 90-day oral toxicity study in rodents
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.302.

EC B.27 - Sub-chronic oral toxicity test: repeated dose 90-day oral toxicity study in non-rodents
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.308.

EC B.28 - Sub-chronic dermal toxicity study: 90-day repeated dermal dose study using rodent species
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.314.

EC B.29 - Sub-chronic inhalation toxicity study: 90-day repeated inhalation dose study using rodent species
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.318.

EC B.30 - Chronic toxicity test
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.323.

EC B.31 - Prenatal developmental toxicity study
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.329.

EC B.32 – Carcinogenicity test
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.338.

EC B.33 - Combined chronic toxicity / carcinogenicity test
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.344.

EC B.35 - Two-generation reproduction toxicity test
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.355.

EC B.36 – Toxicokinetics
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.365.

EC B.40 - *In* vitro skin corrosion: Transcutaneous Electrical Resistance test (TER)
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.384.

EC B.40bis - *In* vitro skin corrosion: Human skin model test
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.394.

EC B.41 - *In vitro* 3T3 NRU phototoxicity test
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.400.

EC B.42 - Skin sensitisation: Local Lymph Node Assay
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.414.

EC B.44 - Skin absorption: *In vivo* method
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.432.

EC B.45 - Skin absorption: *In vitro* method
Council Regulation (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L142, 31/05/2008, p.438.

EC B.46 - *In vitro* skin irritation: Reconstructed human epidermis model test
Commission Regulation (EC) No 761/2009 of 23 July 2009 amending, for the purpose of its adaptation to technical progress, Regulation (EC) No 440/2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).
Official Journal L220, 24/08/2009, p.24.

ECB (European Chemicals Bureau)
Technical Guidance Document on Risk Assessment in support of Commission Directive 93/67/EEC on Risk Assessment for new notified substances, Commission Regulation (EC) No 1488/94 on Risk Assessment for existing substances and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market.
Doc. EUR 20418 EN/1, European Communities (2003).

ECETOC
Percutaneous absorption. European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), Monograph No 20, Brussels (1993).

ECETOC
Recognition of, and Differentiation between, Adverse and Non-adverse Effects in Toxicology Studies. European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), Technical Report No. 85, Brussels (2002).

EChA 2008a. Guidance on information requirements and chemical safety assessment. Chapter R.7a: Endpoint specific guidance. Available through:
http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_en.htm?time=1285848663#r7 (consulted December 2010).

EChA 2008b. Guidance on information requirements and chemical safety assessment. Chapter R.8: Characterisation of dose [concentration]-response for human health.
Available through:
http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_r8_en.pdf?vers=20_08_08 (consulted December 2010).

EFSA (European Food Safety Authority)
Opinion of the Scientific Committee on a request from EFSA related to A Harmonised Approach for Risk Assessment of Substances Which are both Genotoxic and Carcinogenic
The EFSA Journal 282, 1-31 (2005).

EFSA (European Food Safety Authority)
Guidance of the Scientific Committee on Use of the benchmark dose approach in risk assessment
The EFSA Journal 1150, 1-72 (2008).

EMEA 2009.
Note for Guidance on Photosafety Testing. The European Agency for the Evaluation of Medicinal Products, Evaluation of Medicines for Human Use
Available through
http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003353.pdf, consulted December 2010.

ESAC Statement on the application of the Corrositex™ assay for skin corrosivity testing.
Adopted by the ECVAM Scientific Advisory Committee (ESAC) at its 15th Meeting of 5-6 December 2000 at ECVAM, Ispra, Italy (2000).

ESAC Statement on the scientific validity of cytotoxicity/cell-function based *in vitro* assays for eye irritation testing.
Adopted by the ECVAM Scientific Advisory Committee (ESAC) at its 31st Meeting of 7-8 July 2009 at ECVAM, Ispra, Italy (2009).

ESAC Statement on the scientific validity of the Embryonic Stem Cell Test (EST), the Micromass Test and the Postimplantation Rat Whole-Embryo Culture Assay - *in vitro* tests for embryotoxicity. *Adopted by the ECVAM Scientific Advisory Committee (ESAC) at its 17th Meeting of 16-17 October 2001 at ECVAM, Ispra, Italy* (2001).

EUR 10305: Reports of the Scientific Committee on Cosmetology. Fourth series. 1986.

EUR 11080: Reports of the Scientific Committee on Cosmetology. Fifth series. 1987.

EUR 11139: Reports of the Scientific Committee on Cosmetology. Sixth series. 1987.

EUR 11303: Reports of the Scientific Committee on Cosmetology. Seventh series. 1988.

EUR 7297: Reports of the Scientific Committee on Cosmetology. First series. 1982.

EUR 8634: Reports of the Scientific Committee on Cosmetology. Second series. 1983.

EUR 8794: Reports of the Scientific Committee on Cosmetology. Third series. 1983.

European Commission, DG Health and Consumer Protection.
First Report on the Harmonisation of Risk Assessment Procedures, Part 1 . The Report of the Scientific Steering Committee's Working Group on Harmonisation of Risk Assessment Procedures in the Scientific Committees advising the European Commission in the area of human and environmental health (2000), *published on the Internet 20.12.2000*.

European Pharmacopoeia
4th Edition, Supplement 4.2, Council of Europe, Strassbourg (2001).

Ferrario V.F., Sforza C., Schmitz J.H., Ciusa V., Colombo A.
Normal growth and development of the lips: a 3-dimensional study from 6 years to adulthood using a geometric model.
Journal of Anatomy 196(3):415-23 (2000).

Gago-Dominguez M., Castelao J.E., Yuan J.M., Yu M.C., Ross R.K.
Use of permanent hair dyes and bladder-cancer risk.
International Journal on Cancer 91(4), 575-579 (2001).

Gelbke H.P., Kayser M., Poole A.
OECD test strategies and methods for endocrine disruptors.
Toxicology 205(1-2), 17-25 (2004).

Gelbke H.P., Hofmann A., Owens J.W., Freyberger A.
The enhancement of the subacute repeat dose toxicity test OECD TG 407 for the detection of endocrine active chemicals: comparison with toxicity tests of longer duration.
Archives of Toxicology 81(4), 227-250 (2007).

Gilleron L., Coecke S., Sysmans E., Hansen E., Van Oproy S., Marzin D., Van Cauteren H and Vanparys P.
Evaluation of a modified HET-CAM assay as a screening test for eye irritancy.
Toxicology *In Vitro*, 11, 641-644 (1997).

Gocke E., Albertini S., Chetelat A., Kirchner S., Muster W.
The photomutagenicity of fluoroquinolones and other dyes.
Toxicology Letters 102/103, 375-381 (1998).

Gocke E., Müller L., Guzzie P.J., Brendler-Schwaab S., Bulera S., Chignell C.F., Henderson L.M., Jacobs A., Murli H., Snyder R.D., Tanaka N.
Considerations on photochemical genotoxicity. Report of the IWGTP working group.
Environmental Molecular Mutagenicity 35, 173-184 (2000).

Hall B., Tozer S., Safford B., Coroama M., Steiling W., Leneveu-Duchemin M.C., McNamara C., Gibney M.
European consumer exposure to cosmetic products, a framework for conducting population exposure assessments.
Food and Chemical Toxicology 45(11):2097-108 (2007).

Hall B., Steiling W., Safford B., Coroama M., Tozer S., Firmani C., McNamara C., Gibney M.
European consumer exposure to cosmetic products, a framework for conducting population exposure assessments, Part 2.
Food and Chemical Toxicology 49:407-21 (2011).

Harvey P.W. and Everett D.J.
Regulation of endocrine-disrupting chemicals: critical overview and deficiencies in toxicology and risk assessment for human health.
Best Practice & Research Clinical Endocrinology & Metabolism 20(1), 145-65 (2006).

Howes D., Guy R., Hadgraft J., Heylings J., Hoeck U., Kemper F., Maibach H., Marty J-P., Merk H., Parra J., Rekkas D., Rondelli I., Schaefer H., Täuber U. and Verbiese N.
Methods for assessing percutaneous absorption, ECVAM Workshop Report n. 13.
Alternatives To Laboratory Animals 24, 81-106 (1996).

Hotchkiss A.K., Rider C.V., Blystone C.R., Wilson V.S., Harttig P.C., Foster P.M., Gray C.L., Gray L.E.
Fifteen years after „Wingspread" – Environmental endocrine disruptors and human and wildlife health: where we are today and where we need to go.
Toxicological Sciences 105(2), 235-259 (2008).

IARC 2010.
International Agency for Research on Cancer - Monographs: Volume 99 Some Aromatic Amines, Organic Dyes, and Related Exposures (2010). Available through http://monographs.iarc.fr/ENG/Monographs/PDFs/index.php (consulted December 2010).

IGHRC 2006.
Guidelines on route-to-route extrapolation of toxicity data when assessing health risks of chemicals.
The Interdepartmental Group on Health Risks from Chemicals, http://www.silsoe.cranfield.ac.uk /ieh/ighrc/ighrc.html

IP/06/1047: European Commission Press Release
Commission bans 22 hair dye substances to increase consumer safety
Brussels, 20 July 2006, available through *http://europa.eu/rapid/* (consulted September 2006)

JRC (Joint Research Centre)
Draft Report on Alternative (Non-Animal) Methods for Cosmetics Testing: current status and future prospects - 2010. Available through http://ec.europa.eu/consumers/sectors/cosmetics/files/pdf/animal_testing/consultation_animal_testing_en.pdf, consulted December 2010.

Kersten B., Kasper P., Brendler-Schwaab S.Y., Muller L.
Use of the photo-micronucleus assay in Chinese hamster V79 cells to study photochemical genotoxicity.
Mutation Research 519, 49-66 (2002).

Kimber I., Dearman R.J., Betts C.J., Gerberick G.F., Ryan C.A., Kern P.S., Pavlewicz G.Y. and Basketter D.A. The Local Lymph Node Assay and skin sensitisation: a cut-down screen to reduce animal requirements? Contact Dermatitis 54, 181-185 (2006).

Kirkland D., Henderson L., Marzin D., Müller L., Parry J.M., Speit G., Tweats D.J., Williams G.M.
Testing strategies in mutagenicity and genetic toxicology: an appraisal of the guidelines of the European Scientific Committee for Cosmetics and Non-Food Products for the evaluation of hair dyes.
Mutation Research 588(2), 88-105 (2005).

Kirkland D., Reeve L., Gatehouse D., Vanparys P. A core *in vitro* genotoxicity battery comprising the Ames test plus the *in vitro* micronucleus test is sufficient to detect rodent carcinogens and *in vivo* genotoxins.
Mutation Research 721(1), 27-73 (2011).

Kroes R., Galli C., Munro I., Schilter B., Tran L., Walker R., Wurtzen G.
Threshold of toxicological concern for chemical substances present in the diet: a practical tool for assessing the need for toxicity testing.
Food Chem. Toxicol. 38(2-3), 255-312 (2000).

Kroes R., Renwick A.G., Cheeseman. M., Kleiner. J., Mangelsdorf I., Piersma A., Schilter B., Schlatter J., van Schothorst F., Vos J.G., Wurtzen G.
European branch of the International Life Sciences Institute
Structure-based thresholds of toxicological concern (TTC): guidance for application to substances present at low levels in the diet.
Food Chem. Toxicol. 42(1), 65-83 (2004).

Lynch I., Dawson K.A.
Protein-nanoparticle interactions.
Nano Today 3:40-7 (2008).

Macfarlane M., Jones P., Goebel C., Dufour E., Rowland J., Araki D., Costabel-Farkas M., Hewitt N.J., Hibatallah J., Kirst A., McNamee P., Schellauf F., Scheel J.
A tiered approach to the use of alternatives to animal testing for the safety assessment of cosmetics: skin irritation.
Regulatory Toxicology and Pharmacology 54(2):188-96 (2009).

Marx-Stoelting P., Adriaens E., Ahr HJ., Bremer S., Garthoff B., Gelbke H.P., Piersma A., Pellizzer C., Reuter U., Rogiers V., Schenk B., Schwengberg S., Seiler A., Spielmann H., Steemans M., Stedman D.B., Vanparys P., Vericat J.A., Verwei M., van der Water F., Weimer M., Schwarz M.
A review of the implementation of the embryonic stem cell test (EST). The report and recommendations of an ECVAM/ReProTect Workshop.
ATLA 37(3):313-28 (2009).

Matsushima T., Sugimura T., Nagao M., Yahagi T., Shirai A., Sawamura M.
Factors modulating mutagenicity microbial tests.
In: Short-term test systems for detecting carcinogens (Eds. Norpoth K.H. and Garner R.C.)
Springer, Berlin-Heidelberg-New York, 273-285 (1980).

McEwen G.N., Curry A.S. and Graf J.G. (Editors)
Technical Guidelines. Microbiological Guidelines.
CTFA Cosmetic, Toiletries and Fragrance Association, Washington DC, November 2001.

McNamara C., Rohan D., Golden D., Gibney M., Hall B., Tozer S., Safford B., Coroama M., Leneveu-Duchemin M.C., Steiling W. (2007).
Probabilistic modelling of European consumer exposure to cosmetic products.
Food and Chemical Toxicology 45(11):2086-96.

McNamee P., Hibatallah J., Costabel-Farkas M., Goebel C., Araki D., Dufour E., Hewitt N.J., Jones P., Kirst A., Le Varlet B., Macfarlane M., Marrec-Fairley M., Rowland J., Schellauf F., Scheel J.
A tiered approach to the use of alternatives to animal testing for the safety assessment of cosmetics: eye irritation.
Regulatory Toxicology and Pharmacology 54(2):197-209 (2009).

Miller B., Potter-Locher F., Seelbach A., Stopper H., Utesch D., Madle S.
Evaluation of the *in vitro* micronucleus test as an alternative to the *in vitro* chromosomal aberration assay: position of the GUM Working Group on the *in vitro* micronucleus test. Gesellschaft fur Umwelt-Mutations-forschung.
Mutation Research 10(1):81-116 (1998).

Muller L., Blakey D., Dearfield K.L., Galloway S., Guzzie P., Hayashi M., Kasper P., Kirkland D. MacGregor J.T., Parry J.M., Schechtman L., Smith A., Tanaka N., Tweats D., Yamasaki H.
Strategy for genotoxicity testing and stratification of genotoxicity test results- report on initial activities of the IWGT Expert Group.
Mutation Research 540, 177-181 (2003).

OECD
OECD Guideline for testing of chemicals - Draft Guideline: *In vitro* Syrian Hamster Embryo (SHE) Cell Transformation Assay
Organization for Economic Cooperation and Development, Paris, draft approved May 1996.

OECD
Guidelines for the Testing of Chemicals: Introduction to the OECD Guidelines on Genetic Toxicology Testing and Guidance on the Selection and Application of Assays Vol.2, pp.1-18, Paris (2000).

OECD
Guidance Document for the Conduct of Skin Absorption Studies. Document number ENV/JM/MONO(2004)2. Organization for Economic Cooperation and Development (OECD), Environment Directorate, OECD Environmental Health and Safety Publications, Series on Testing and Assessment No. 28, Paris, 5 March 2004.

OECD
Guideline for the Testing of Chemicals. Draft proposal for a new Guideline: *In Vitro* Skin Irritation: Reconstructed Human Epidermis (RHE) Test Method.
Organization for Economic Cooperation and Development, Paris, version 11 December 2009.

OECD
OECD Working Party on Manufactured Nanomaterials, Preliminary review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials, ENV/CHEM/NANO(2009)6/REV1, Environment Directorate Organisation For Economic Co-Operation and Development Paris, 2009.

OECD 401 - OECD Guideline for testing of chemicals - Guideline 401: Acute Oral Toxicity
Organization for Economic Cooperation and Development, Paris, adopted 12 May 1981, last updated 24 February 1997 and deleted 17 December 2002.

OECD 402 - OECD Guideline for testing of chemicals - Guideline 402: Acute Dermal Toxicity
Organization for Economic Cooperation and Development, Paris, adopted 24 February 1997.

OECD 404 - OECD Guideline for testing of chemicals - Guideline 404: Acute Dermal Irritation/Corrosion
Organization for Economic Cooperation and Development, Paris, adopted 12 May 1981, last updated 24 April 2002.

OECD 405 - OECD Guideline for testing of chemicals - Guideline 405: Acute Eye Irritation/Corrosion
Organization for Economic Cooperation and Development, Paris, adopted 12 May 1981, last updated 24 April 2002.

OECD 406 - OECD Guideline for testing of chemicals - Guideline 406: Skin Sensitisation
Organization for Economic Cooperation and Development, Paris, adopted 17 July 1992.

OECD 407 - OECD Guideline for testing of chemicals - Guideline 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents
Organization for Economic Cooperation and Development, Paris, adopted 3 October 2008.

OECD 408 - OECD Guideline for testing of chemicals - Guideline 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents
Organization for Economic Cooperation and Development, Paris, adopted 21 September 1998.

OECD 409 - OECD Guideline for testing of chemicals - Guideline 409: Repeated Dose 90-Day Oral Toxicity Study in Non-Rodents
Organization for Economic Cooperation and Development, Paris, adopted 21 September 1998.

OECD 410 - OECD Guideline for testing of chemicals - Guideline 410: Repeated Dose Dermal Toxicity: 21/28-Day Study
Organization for Economic Cooperation and Development, Paris, adopted 12 May 1981.

OECD 411 - OECD Guideline for testing of chemicals - Guideline 411: Subchronic Dermal Toxicity: 90-Day Study
Organization for Economic Cooperation and Development, Paris, adopted 12 May 1981.

OECD 412 - OECD Guideline for testing of chemicals - Guideline 412: Subacute Inhalation Toxicity: 28-Day Study
Organization for Economic Cooperation and Development, Paris, adopted 7 September 2009.

OECD 413 - OECD Guideline for testing of chemicals - Guideline 413: Subchronic Inhalation Toxicity: 90-Day Study
Organization for Economic Cooperation and Development, Paris, adopted 7 September 2009.

OECD 414 - OECD Guideline for testing of chemicals - Guideline 414: Prenatal Developmental Toxicity Study
Organization for Economic Cooperation and Development, Paris, adopted 22 January 2001.

OECD 416 - OECD Guideline for testing of chemicals - Guideline 416: Two-Generation Reproduction Toxicity Study
Organization for Economic Cooperation and Development, Paris, adopted 26 May 1983, last updated 22 January 2001.

OECD 417 - OECD Guideline for testing of chemicals - Guideline 417: Toxicokinetics
Organization for Economic Cooperation and Development, Paris, adopted 4 April 1984, last updated 22 July 2010.

OECD 420 - OECD Guideline for testing of chemicals - Guideline 420: Acute Oral Toxicity - Fixed Dose Procedure
Organization for Economic Cooperation and Development, Paris, adopted 17 July 1992, last updated 17 December 2001.

OECD 421 - OECD Guideline for testing of chemicals - Guideline 421: Reproduction/Developmental Toxicity Screening Test
Organization for Economic Cooperation and Development, Paris, adopted 27 July 1995.

OECD 423 - OECD Guideline for testing of chemicals - Guideline 423: Acute Oral toxicity - Acute Toxic Class Method
Organization for Economic Cooperation and Development, Paris, adopted 22 March 1996, last updated 17 December 2001.

OECD 425 - OECD Guideline for testing of chemicals - Guideline 425: Acute Oral Toxicity: Up-and-Down-Procedure
Organization for Economic Cooperation and Development, Paris, adopted 3 October 2008.

OECD 427 - OECD Guideline for testing of chemicals - Guideline 427: Skin absorption: *In vivo* method
Organization for Economic Cooperation and Development, Paris, adopted 13 April 2004.

OECD 428 - OECD Guideline for testing of chemicals - Guideline 428: Skin absorption: *In vitro* method
Organization for Economic Cooperation and Development, Paris, adopted 13 April 2004.

OECD 429 - OECD Guideline for testing of chemicals - Guideline 429: Skin Sensitisation: Local Lymph Node Assay
Organization for Economic Cooperation and Development, Paris, adopted 24 April 2002.

OECD 430 - OECD Guideline for testing of chemicals - Guideline 430: *In vitro* Skin Corrosion: Transcutaneous Electrical Resistance Test (TER)
Organization for Economic Cooperation and Development, Paris, adopted 13 April 2004.

OECD 431 - OECD Guideline for testing of chemicals - Guideline 431: *In vitro* Skin Corrosion: Human Skin Model Test
Organization for Economic Cooperation and Development, Paris, adopted 13 April 2004.

OECD 432 - OECD Guideline for testing of chemicals - Guideline 432: *In vitro* 3T3 NRU phototoxicity test
Organization for Economic Cooperation and Development, Paris, adopted 13 April 2004.

OECD 435 - OECD Guideline for testing of chemicals - Guideline 435: *In vitro* Membrane Barrier Test Method for Skin Corrosion.
Organization for Economic Cooperation and Development, Paris, adopted 19 July 2006.

OECD 436 - OECD Guideline for testing of chemicals - Guideline 436: Acute Inhalation Toxicity - Acute Toxic Class (ATC) Method.
Organization for Economic Cooperation and Development, Paris, adopted 7 September 2009.

OECD 437 - OECD Guideline for testing of chemicals - Guideline 437: Bovine Corneal Opacity and Permeability Test Method for Identifying Ocular Corrosives and Severe Irritants.
Organization for Economic Cooperation and Development, Paris, adopted 7 September 2009.

OECD 438 - OECD Guideline for testing of chemicals - Guideline 438: Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants.
Organization for Economic Cooperation and Development, Paris, adopted 7 September 2009.

OECD 451 - OECD Guideline for testing of chemicals - Guideline 451: Carcinogenicity Studies
Organization for Economic Cooperation and Development, Paris, adopted 7 September 2009.

OECD 452 - OECD Guideline for testing of chemicals - Guideline 452: Chronic Toxicity Studies
Organization for Economic Cooperation and Development, Paris, adopted 7 September 2009.

OECD 453 - OECD Guideline for testing of chemicals - Guideline 453: Combined Chronic Toxicity/Carcinogenicity Studies
Organization for Economic Cooperation and Development, Paris, adopted 7 September 2009.

OECD 471 - OECD Guideline for testing of chemicals - Guideline 471: Bacterial Reverse Mutation Test
Organization for Economic Cooperation and Development, Paris, adopted 26 May 1983, last updated 21 July 1997.

OECD 473 - OECD Guideline for testing of chemicals - Guideline 473: *In vitro* Mammalian Chromosomal Aberration Test
Organization for Economic Cooperation and Development, Paris, adopted 21 July 1997.

OECD 476 - OECD Guideline for testing of chemicals - Guideline 476: *In vitro* Mammalian Cell Gene Mutation Test
Organization for Economic Cooperation and Development, Paris, adopted 21 July 1997.

OECD 482 - OECD Guideline for testing of chemicals - Guideline 482: Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells *in vitro*
Organization for Economic Cooperation and Development, Paris, adopted 23 October 1986.

OECD 487 - OECD Guideline for testing of chemicals - Guideline 487: *In vitro* Mammalian Cell Micronucleus Test (MNvit).
Organization for Economic Cooperation and Development, Paris, adopted 22 July 2010.

OECD 495 - OECD Guideline for testing of chemicals - Draft Guideline 495: *In vitro* Syrian Hamster Embryo (SHE) Cell Transformation Assay.
Organization for Economic Cooperation and Development, Paris, draft approved, but not on website.

Parry J.M.
A proposal for a new OECD Guideline for the *in vitro* micronucleus test.
EEMS Draft (1997).

Pelkonen O., Coecke S., Batista Leite S., Bernauer U., Bessems J., Brandon E., Bois F., Gundert-Remy U., Loizou G., Testai E., Zaldívar M.
Chapter 4: Toxicokinetics.
In: Draft Report on Alternative (Non-Animal) Methods for Cosmetics Testing: current status and future prospects - 2010. Available through http://ec.europa.eu/consumers/sectors/cosmetics/files/pdf/animal_testing/consultation_animal_testing_en.pdf, consulted December 2010.

Peto R., Pike M.C., Bernstein L., Gold L.S., Ames B.N.
The TD_{50}: a proposed general convention for the numerical description of the carcinogenic potency of chemicals in chronic-exposure animal experiments.
Environmental Health Perspectives 58, 1-8 (1984).

Pflaum M., Kielbassa C., Garmyn M., Epe B.G.
Oxidative DNA damage induced by visible light in mammalian cells; extent, inhibition by antioxidants and genotoxic effects.
Mutation Research 408, 137-144 (1998).

Prival, M.J., Bell S.J., Mitchell V.D., Peiperl M.D., Vaughan V.L.
Mutagenicity of Benzidine and Benzidine-congener dyes and selected monoazo dyes in a modified Salmonella assay.
Mutation Research 136, 33-47 (1984).

Renwick A.G.
Toxicokinetics in infants and children in relation to the ADI and TDI.
Food Additives and Contaminants Suppl. 15, 17-35 (1998).

Rocks S., Pollard S., Dorey R., Levy L., Harrison P., Handy R. Comparison of risk assessment approaches for manufactured nanomaterials, Defra, London (2008).

Rogiers V. and Beken S. (Editors and authors)
Alternative Methods to Animal experiments. Actual status, development and approach in Belgium.
VUBPress, Brussels. ISBN 90-5487-264-0 (2000).

Rogiers V.
Ecopa: a powerful concept in the way forward for alternative methods.
Alternatives To Laboratory Animals 30, Supplement 2, 199-202 (2002).

Rogiers V.
"Validated" and "valid" alternative methods available today for testing of cosmetic products and their ingredients.
In: Safety Assessment of Cosmetics in the EU. Training Course Vrije Universiteit Brussel, 7-12 April 2003, Part 2, p.1.

Rogiers V. and Pauwels M.
Good science must be the key factor in the development and use of alternative methods for safety assessment of cosmetics.
ALTEX 22 (Special Issue), 259 (2005).

Rogiers V.
Hazard, risk, risk assessment and risk perception : key factors in the safety evaluation of cosmetics.
In: *Safety Assessment of Cosmetics in the EU - Training Course, Part I*,
Vrije Universiteit Brussel, 12-17 April 2010, pp.53-70 (2010).

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.

Salminen W.F. Jr.
Integrating toxicology into cosmetic ingredient research and development.
International Journal of Cosmetic Science 24, 217-224 (2002).

Sanner T., Dybing E., Willems M.I. and Kroese E.D.
A simple method for quantitative risk assessment of non-threshold carcinogens based on the dose descriptor T25.
Pharmacol Toxicol 88: 331-341 (2001).

SCCNFP/0003/98: Guidelines on the use of human volunteers in the testing of potentially cutaneous irritant cosmetic ingredients or mixtures of ingredients, *adopted by the plenary session of the SCCNFP of 25 November 1998*.

SCCNFP/0017/98: Opinion concerning fragrance allergy in consumers: a review of the problem. Analysis of the need for appropriate consumer information and identification of consumer allergens, *adopted by the SCCNFP during the plenary session of 8 December 1999*.

SCCNFP/0042/98: The Scientific Committee Cosmetic and Non-Food Products intended for consumers, Rules of Procedure, *adopted on the 16th plenary meeting of the SCCNFP of 13 March 2001.*

SCCNFP/0059/98: Opinion concerning foreseeable use of hair dyes, *adopted by the plenary session of the SCCNFP of 23 September 1998*.

SCCNFP/0068/98: 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/0069/98: Opinion on *in vitro* methods to assess phototoxicity in the safety evaluation of cosmetic ingredients or mixtures of ingredients, *adopted by the SCCNFP during the plenary session of 25 November 1998*.

SCCNFP/0098/99: Status report on the inventory of cosmetic ingredients, *approved by the plenary session of the SCCNFP on 17 February 1999*.

SCCNFP/0099/99: Position paper concerning the present situation of the *Pseudo* INCI names of botanicals, *approved by the plenary session of the SCCNFP on 17 February 1999*.

SCCNFP/0103/99: Opinion on the use of alternative methods to animal testing in the safety evaluation of cosmetic ingredients or mixtures of ingredients, *adopted by the SCCNFP at the plenary meeting of 20 January 1999*.

SCCNFP/0119/99: Notes of Guidance for Testing of Cosmetic Ingredients for their Safety Evaluation. Third Revision. *adopted by the SCCNFP during the plenary meeting of 23 June 1999.*

SCCNFP/0120/99: Opinion concerning the predictive testing of potentially cutaneous sensitising cosmetic ingredients or mixtures of ingredients, *adopted by the SCCNFP during the 11th plenary session of 17 February 2000*.

SCCNFP/0125/99: Restrictions on materials listed in Annex VI of Directive 76/768/EEC on cosmetic products, *adopted by the SCCNFP during the 7th plenary meeting of 17 February 1999.*

SCCNFP/0167/99: Basic Criteria for the *in vitro* assessment of percutaneous absorption of cosmetic ingredients, *adopted by the SCCNFP during the 8th plenary meeting of 23 June 1999.*

SCCNFP/0245/99: 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, *adopted by the SCCNFP during the plenary session of 8 December 1999*.

SCCNFP/0299/00: 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*.

SCCNFP/0320/00: Opinion concerning an initial list of perfumery materials which must not form part of fragrances compounds used in cosmetic products, *adopted by the SCCNFP during the 12th plenary meeting of 3 May 2000*.

SCCNFP/0321/00: 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*.

SCCNFP/0389/00: Opinion concerning the 1st update of the inventory of ingredients employed in cosmetic products. Section II: perfume and aromatic raw materials, *adopted by the SCCNFP during the plenary session of 24 October 2000*.

SCCNFP/0392/00: Opinion concerning an Initial List of Perfumery Materials which must not form part of Cosmetic Products except subject to the restrictions and conditions laid down, *adopted by the SCCNFP during the 18th plenary meeting of 25 September 2001*.

SCCNFP/0450/01: Memorandum on the SCCNFP opinion concerning fragrance allergy in consumers, *adopted by the SCCNFP during the 16th plenary meeting of 16 March 2001*.

SCCNFP/0451/01: Opinion concerning amendment to entry n° 419 of Annex II to Directive 76/768/EEC on Cosmetic Products, *adopted by the SCCNFP during the 17th plenary meeting of 12 June 2001*.

SCCNFP/0461/01: Memorandum on Scientific Evaluations and Opinions, *adopted by the SCCNFP during the 17th plenary meeting of 12 June 2001*.

SCCNFP/0474/01: Opinion concerning chemical ingredients in cosmetic products classified as carcinogenic, mutagenic or toxic to reproduction according to the Chemicals Directive 67/548/EEC, *adopted by the SCCNFP during the 18th plenary meeting of 25 September 2001*.

SCCNFP/0474/01: Opinion concerning chemical ingredients in cosmetic products classified as carcinogenic, mutagenic or toxic to reproduction according to the Chemicals Directive 67/548/EEC, *adopted by the SCCNFP during the 18th plenary meeting of 25 September 2001*.

SCCNFP/0483/01: Opinion on the evaluation of potentially estrogenic effects of UV filters, *adopted by the SCCNFP during the 17th plenary meeting of 12 June 2001*.

SCCNFP/0484/01: Opinion on the use of permanent hair dyes and bladder cancer risk, *adopted by the SCCNFP during the 17th plenary meeting of 12 June 2001*.

SCCNFP/0484/01: Opinion on the use of permanent hair dyes and bladder cancer risk, *adopted by the SCCNFP during the 17th plenary meeting of 12 June 2001*.

SCCNFP/0495/01: Opinion concerning the safety review of the use of certain azo-dyes in cosmetic products, *adopted by the SCCNFP during the 19th plenary meeting of 27 February 2002*.

SCCNFP/0521/01: Opinion concerning amendment to entry n° 419 of Annex II to Directive 76/768/EEC on Cosmetic Products, *adopted by the SCCNFP during the 18th plenary meeting of 25 September 2001*.

SCCNFP/0546/02: Memorandum concerning the actual status of alternative methods to the use of animals in the safety testing of cosmetic ingredients, *adopted by the SCCNFP during the 20th plenary meeting of 4 June 2002*.

SCCNFP/0552/02: Opinion concerning amendment to entry n° 419 of Annex II to Directive 76/768/EEC on Cosmetic Products, *adopted by the SCCNFP during the 19th plenary meeting of 27 February 2002*.

SCCNFP/0553/02: Discussion paper on assessment strategies for hair dyes, *adopted by the SCCNFP during the 19th plenary meeting of 27 February 2002*.

SCCNFP/0557/02: Position statement on the calculation of the Margin of Safety of ingredients incorporated in cosmetics which may be applied to the skin of children, *adopted by the SCCNFP during the 19th plenary meeting of 27 February 2002*.

SCCNFP/0566/02: Proposal for a strategy for testing hair dye cosmetic ingredients for their potential genotoxicity/mutagenicity, *adopted by the SCCNFP during the 20th plenary meeting of 4 June 2002*.

SCCNFP/0612/02: Opinion concerning amendment to entry n° 419 of Annex II to Directive 76/768/EEC on Cosmetic Products, *adopted by the SCCNFP during the 22nd plenary meeting of 17 December 2002*.

SCCNFP/0633/02: Updated Basic Requirements for toxicological dossiers to be evaluated by the SCCNFP, *adopted by the SCCNFP during the 22nd plenary meeting of 17 December 2002*.

SCCNFP/0635/03: Opinion concerning request for a re-evaluation of hair dyes listed in Annex III to Directive 76/768/EEC on Cosmetic Products, *adopted by the SCCNFP during the 23rd plenary meeting of 18 March 2003*.

SCCNFP/0657/03: Opinion concerning Dihydroxyindole (Colipa n° A111), *adopted by the SCCNFP during the 23rd plenary meeting of 18 March 2003*.

SCCNFP/0669/03: Opinion concerning Dihydroxyindoline HBr (Colipa n° A147), *adopted by the SCCNFP during the 23rd plenary meeting of 18 March 2003*.

SCCNFP/0690/03: Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation, *adopted by the SCCNFP during the 25th plenary meeting of 20 October 2003*.

SCCNFP/0720/03: Updated recommended strategy for testing hair dyes for their potential genotoxicity/mutagenicity/carcinogenicity, *adopted by the SCCNFP during the 24th plenary meeting of 24-25 June 2003*.

SCCNFP/0720/03: Updated recommended strategy for testing hair dyes for their potential genotoxicity/mutagenicity/carcinogenicity, *adopted by the SCCNFP during the 24th plenary meeting of 24-25 June 2003*.

SCCNFP/0724/03: Opinion concerning use of specified risk materials in cosmetics: clarification for tallow derivatives, *adopted by the SCCNFP by written procedure on 23 july 2003*.

SCCNFP/0750/03: Basic Criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients - updated November 2003,
adopted by the SCCNFP during the 25th plenary meeting of 20 October 2003.

SCCNFP/0753/03: Consultation concerning risks and health effects from tattoos, body piercing and related practices, *adopted by the SCCNFP during the 25th plenary meeting of 20 October 2003*.

SCCNFP/0770/03: Opinion concerning an update of the initial list of perfumery materials which must not form part of cosmetic products except subject to the restrictions and conditions laid down, *adopted by the SCCNFP during the 26th plenary meeting of 9 December 2003*.

SCCNFP/0771/03: Opinion concerning an update of the initial list of perfumery materials which must not form part of fragrance compounds used in cosmetic products, *adopted by the SCCNFP during the 26th plenary meeting of 9 December 2003*.

SCCNFP/0797/04: Opinion concerning use of permanent hair dyes and bladder cancer, *adopted by the SCCNFP on 23 April 2004 by means of the written procedure*.

SCCNFP/0807/04: Opinion concerning hair dyes without file submitted, *adopted by the SCCNFP on 23 April 2004 by means of the written procedure*.

SCCNFP/0808/04: Opinion concerning ring study on reaction products from typical combinations of hair colouring ingredients, *adopted by the SCCNFP on 23 April 2004 by means of the written procedure*.

SCCNFP/0825/04: Opinion concerning chemical ingredients in cosmetic products classified as carcinogenic, mutagenic or toxic to reproduction according to the Chemicals Directive 67/548/EEC, *adopted by the SCCNFP during the 28th plenary meeting of 25 May 2004*.

SCCNFP/0834/04: Opinion concerning "Report for establishing the timetable for phasing out animal testing for the purpose of the cosmetics directive" issued by ECVAM (30/04/2004), *adopted by the SCCNFP on 1 July 2004 by means of the written procedure*.

SCCP, 2006: Memorandum on hair dye substances and their skin sensitising properties, *adopted by the SCCP during the 10h plenary meeting of 19 December 2006*.

SCCP/0851/04: Opinion on the safety of Triclocarban (Colipan° P29) for other uses than as a preservative, *adopted by the SCCP by written procedure on 1 June 2005*.

SCCP/0882/05: Opinion on the safety of fluorine compounds in oral hygiene products for children under the age of 6 years, *adopted by the SCCP during the 5th plenary meeting of 20 September 2005*.

SCCP/0888/05: Opinion concerning request for confirmation of the SCCNFP opinion 0474/01 on chemical ingredients in cosmetic products classified as carcinogenic, mutagenic or toxic to reproduction according to Council Directive 67/548/EEC, *adopted by the SCCP during the 3rd plenary meeting of 15 March 2005*.

SCCP/0894/05: Opinion on amino acids obtained by hydrolysis of human hair, *adopted by the SCCP during the 4th plenary meeting of 21 June 2005*.

SCCP/0913/05: Opinion concerning request for confirmation of the SCCNFP opinion 0474/01 on chemical ingredients in cosmetic products classified as carcinogenic, mutagenic or toxic to reproduction according to Council Directive 67/548/EEC, *adopted by the SCCP during the 4th plenary meeting of 21 June 2005*.

SCCP/0919/05: Memorandum on the classification and categorization of skin sensitisers and grading of test reactions, *adopted by the SCCP during the 5th plenary meeting of 20 September 2005*.

SCCP/0930/05: Opinion on personal use of hair dyes and cancer risk, *adopted by the SCCP during the 5th plenary meeting of 20 September 2005*.

SCCP/0933/05: Opinion on risk of ingredients deriving from category 1-material and category 2-material as defined in Regulation 1774/2002 in cosmetic products, *adopted by the SCCP during the 5th plenary meeting of 20 September 2005*.

SCCP/0941/05: Opinion on exposure to reactants and reaction products of oxidative hair dye formulations, *adopted by the SCCP during the 6th plenary meeting of 13 December 2005*.

SCCP/0949/05: Opinion on biological effects of ultraviolet radiation relevant to health with particular reference to sunbeds for cosmetic purposes, *adopted by the SCCP during the 8th plenary meeting of 20 June 2006*.

SCCP/0959/05: Review of the SCCNFP opinion on Hair Dye Strategy in the light of additional information, *adopted by the SCCP during the 8th plenary meeting of 20 June 2006*.

SCCP/0970/06: Opinion on basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients - updated February 2006, *adopted by the SCCP during the 7th plenary meeting of 28 March 2006*.

SCCP/0971/06: Updated recommended strategy for testing oxidative hair dye substances for their potential mutagenicity/genotoxicity*, adopted by the SCCP during the 7th plenary meeting of 28 March 2006*.

SCCP/0974/06: Guidance document on epidemiological and clinical studies on tooth whitening products, *adopted by the SCCP during the 7th plenary meeting of 28 March 2006*.

SCCP/0989/06: Opinion on p-Phenylenediamine (Colipa n° A7), *adopted by the SCCP during the 9th plenary meeting of 10 October 2006*.

SCCP/1004/06: Skin penetration of oxidative hair dyes formed by the coupling of precursors and couplers under simulated conditions of hair dyeing - update of the Annex to the Opinion on Exposure to reactants and reaction products of oxidative hair dye formulations (doc. n° SCCP/0941/05), *adopted by the SCCP during the 8th plenary meeting of 20 June 2006*.

SCCP/1017/06: Opinion on Parabens (Colipa n° P82), *adopted by the SCCP during the 9th plenary meeting of 10 October 2006*.

SCCP/1023/06: Opinion on clarifications to SCCNFP/0392/00 "An initial list of perfumery materials which must not form part of cosmetic products except subject to the restrictions and conditions laid down"*, adopted by the SCCP during the 8th plenary meeting of 20 June 2006*.

SCCP/1086/07: Opinion on Homosalate (Colipa n° S12), *adopted by the SCCP during the 11th plenary meeting of 21 March 2007*.

SCCP/1104/07: Opinion on sensitivity to hair dyes - consumer self testing, *adopted by the SCCP during the 14h plenary meeting of 18 December 2007*.

SCCP/1111/07: Memorandum on actual status of alternative methods on the use of experimental animals in the safety assessment of cosmetic ingredients in the European Union, *adopted by the SCCP during the 12h plenary meeting of 19 June 2007*.

SCCP/1145/07: Memorandum on the *in vitro* test EPISKIN™ for skin irritation testing, *adopted by the SCCP during the 14h plenary meeting of 18 December 2007*.

SCCP/1147/07: Opinion on safety of nanomaterials in cosmetic products, *adopted by the SCCP after the public consultation during the 14h plenary meeting of 18 December 2007*.

SCCP/1153/08: Opinion on dermal sensitisation quantitative risk assessment (citral, farnesol and phenylacetaldehyde), *adopted by the SCCP during the 16h plenary meeting of 24 June 2008.*

SCCP/1171/08: SCHER/SCCP/SCENIHR scientific opinion on the use of the Threshold of Toxicological Concern (TTC) approach for the safety assessment of chemical substances - Preliminary report, *agreed by SCHER, SCCP and SCENIHR on 19 November 2008 by written procedure*.

SCCP/1183/08: Opinion on 4-Methylbenzylidene camphor, 4-MBC (Colipa n° P82), *adopted by the SCCP during the 16th plenary of 24 June 2008*.

SCCP/1184/08: Opinion on Parabens (Colipa n° S60), *adopted by the SCCP during the 16th plenary of 24 June 2008*.

SCCP/1192/08: Opinion on Triclosan (Colipa n° P32), *adopted by the SCCP during the 19th plenary meeting of 21 January 2009*.

SCCP/1212/09: Position statement on genotoxicity / mutagenicity testing of cosmetic ingredients without animal experiments, *adopted by the SCCP during the 19h plenary meeting of 21 January 2009*.

SCCP/1214/09: Clarification on the opinions SCCNFP/0653/03 and SCCP/0882/05 on the safety of fluorine compounds in oral hygiene products for children under the age of 6 years, *adopted by the SCCP during the 19th plenary meeting of 21 January 2009*.

SCCS/1241/10: Opinion on Cyclomethicone Octamethylcyclotetrasiloxane (Cyclotetrasiloxane, D4) and Decamethyl-cyclopentasiloxane (Cyclopentasiloxane, D5), *adopted by the SCCS during the 7th plenary meeting of 22 June 2010*.

SCCS/1284/09: Opinion on the new classification of substances as carcinogenic, mutagenic or toxic to reproduction according to the Commission Regulation 790/2009, *adopted by the SCCS during the 5th plenary meeting of 8 December 2009*.

SCCS/1294/10: Memorandum on alternative test methods in human health safety assessment of cosmetic ingredients in the European Union, *adopted by the SCCS during the 5th plenary meeting of 8 December 2009*.

SCCS/1311/10: Opinion on reaction products of oxidative hair dye ingredients formed during hair dyeing processes, *adopted by the SCCS during the 8th plenary meeting of 21 September 2010*.

SCCS/1347/10: Opinion on Dihydroxyacetone, *adopted by the SCCS during the 9th plenary meeting of 14 December 2010.*

SCCS/1358/10: Basic criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients, *adopted by the SCCS during the 7th plenary meeting of 22 June 2010*.

SCCS/1392/10: Memorandum (addendum) on the *in vitro* test EPISKIN™ for skin irritation testing, *adopted by the SCCS during the 9th plenary meeting of 14 December 2010.*

SCENIHR/002/05: Modified opinion (after public consultation) on the appropriateness of existing methodologies to assess the potential risks associated with engineered and adventitious products of nanotechnologies, *adopted by the SCENIHR during the 10th plenary meeting of 10 March 2006.*

SCENIHR
Risk Assessment of Products of Nanotechnologies, *adopted by the SCENIHR during the 28th plenary meeting of 19 January 2009.*

Schaefer H. and Redelmeier T.E.
Skin Barrier, Principles of Percutaneous Absorption, Karger, Basel (1996).

SCHER/SCCP/SCENIHR, 2009 - Scientific opinion on risk assessment methodologies and approaches for genotoxic and carcinogenic substances, *adopted on the 19th plenary meeting of the SCCP of 21 January 2009.*

Schlumpf M., Cotton B., Conscience M., Haller V., Steinmann B. and Lichtensteiger W.
In vitro and *in vivo* estrogenicity of UV screens.
Environmental Health Perspectives 109(3), 239-244 (2001).

Scott L., Eskes C., Hoffmann S., Adriaens E., Alepée N., Bufo M., Clothier R., Facchini D., Faller C., Guest R., Harbell J., Hartung T., Kamp H., Varlet B.L., Meloni M., McNamee P., Osborne R., Pape W., Pfannenbecker U., Prinsen M., Seaman C., Spielmann H., Stokes W., Trouba K., Berghe C.V., Van Goethem F., Vassallo M., Vinardell P., Zuang V.
A proposed eye irritation testing strategy to reduce and replace *in vivo* studies using Bottom-Up and Top-Down approaches.
Toxicology In Vitro 24(1):1-9 (2010).

SCs/01/04 final (C7(2004)D/370235): The Scientific Committees on Consumer Products (SCCP), Health and Environmental Risks (SCHER) and Emerging and Newly Identified Health Risks (SCENIHR): Rules of Procedure,
adopted by DG SANCO (C7 - Risk Assessment) on 7 September 2004.

Šimon P., Joner E.
Conceivable interactions of biopersistent nanoparticles with food matrix and living systems following from their physicochemical properties
Journal of Food and Nutrition Research, 47:51-59 (2008).

SPC/803/5/90: Notes of Guidance for the toxicity testing of cosmetic ingredients.
1st revision, 1990.

Spielmann H., Balls M., Dupuis J., Pape W.J.W., De Silva O., Holzhütter H.G., Gerberick F., Liebsch M., Lowell W.W., and Pfannenbecker V.
A Study on UV filter chemicals from Annex VII of the European Union Directive 76/768/EEC in the *in vitro* NRU Phototoxicity test.
Alternatives To Laboratory Animals 26, 679-708 (1998)

US EPA (Environmental Protection Agency).
Guidelines for Carcinogen Risk Assessment.
Federal Register 51, 33992-34003 (1986).

US EPA
Health Effect Test Guidelines. Dermal Penetration. US Environmental Protection Agency (EPA).
Doc. EPA 712-C-96-350, Washington, DC (1996a).

US EPA (Environmental Protection Agency).
Proposed guidelines for carcinogen risk assessment.
Fed. Reg. 61, 17960-18011 (1996b).

US EPA
Exposure Factors Handbook
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.

US Pharmacopoeia
25th Edition, Monograph 1111 (2002).

Van Benthem J., Felter S., Heinonen T., Poth A., Serafimova R., van Delft J., Benfenati E.; Phrakonkham P., Worth A., Corvi R.
Chapter 3: Carcinogenicity.
In: Draft Report on Alternative (Non-Animal) Methods for Cosmetics Testing: current status and future prospects - 2010. Available through http://ec.europa.eu/consumers/sectors/cosmetics/files/pdf/animal_testing/consultation_animal_testing_en.pdf, consulted December 2010.

Vandebriel R.J., van Loveren H.
Non-animal sensitization testing: state-of-the-art.
Critical Reviews in Toxicology 40(5):389-404 (2010).

WHO (World Health Organization)
Assessing human health risks of chemicals: derivation of guidance values for health-based exposure limits.
Environmental Health Criteria, 170, WHO, Geneva (1994).

WHO (World Health Organization)
Principles for the assessment of Risks to human health from exposure to chemicals.
Environmental Health Criteria, 210, WHO, Geneva (1999).

WHO (World Health Organisation)
Kielhorn J., Melching-Kollmuβ S., Mangelsdorf I.
Dermal Absorption.
WHO / IPCS Environmental Health Criteria, Draft February 2005, accessible through http://www.who.int/ipcs/methods/dermal_absorption/en/ (consulted December 2010).

WHO / UNEP / ILO
Approaches to Integrated Risk Assessment, Doc. WHO/IPCS/IRA/01/12 of December 2001 (2001).

Worth A.P. and Balls M.
Alternative (Non-animal) Methods for Chemicals Testing: Current Status and Future Prospects. A Report prepared by ECVAM and the ECVAM working Group on Chemicals.
Alternatives To Laboratory Animals 30 Supplement 1, 1-125 (2002).

Worth A.P. and Balls M.
The importance of the prediction model in the development and validation of alternative tests.
Alternatives To Laboratory Animals 29, 135-143 (2001).

XXIV/1878/97: Notes of Guidance for Testing of Cosmetic Ingredients for their Safety Evaluation. 2nd revision, 1997.

XXIV/1895/98: Opinion concerning hypoallergenic claims on cosmetic products, *adopted by the plenary session of the SCCNFP of 20 May 1998*.

APPENDIX 1: LISTS OF INGREDIENTS

1. INTRODUCTION

Regulated cosmetic ingredients can be found as Annexes II, III, IV, VI and VII to Directive 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 Cosmetic Ingredient) inventory [96/335/EC], identifying a large number of substances with their possible function(s) in finished cosmetic products and with the nomenclature that needs to be used on the label of finished cosmetic products. DG Sanco has built up a free to use database of cosmetic ingredients called **CosIng**, which combines INCI names and synonyms of the listed ingredients with useful regulatory information.

Finally, this chapter briefly mentions Annex I to the Dangerous Substances legislation [67/548/EEC], since the "7th Amendment" of Directive 76/768/EEC [2003/15/EC] directly refers to that list when excluding CMR Cat.1 & Cat.2 chemicals from cosmetic use (see 3-6.6). With the new European Regulation on classification and labelling [2008/1272/EC], however, Annex I to Dir. 67/548/EEC now needs to be referred to as 'Part 3 of Annex VI to Regulation (EC) No 1272/2008', in which all existing European classifications are converted into new harmonised classifications using the new criteria.

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

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 colorants 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 Directive 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 SCCS / SCC(NF)P.

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

3. INVENTORY OF INGREDIENTS USED IN COSMETIC PRODUCTS

Article 5a of Directive 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, part of which amended by 2006/257/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 authorised 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
- Personal Care Products Council (PCPC) name
- European Pharmacopoeia (Ph. Eur.) name
- International Non-proprietary Name (INN) name, recommended by WHO
- International Union of Pure and Applied Chemistry (IUPAC) name
- EC number, meaning either:
  European INventory of Existing commercial Chemical Substances (EINECS) number (format 2xx-xxx-x)
  European LIst of Notified Chemical Substances (ELINCS) number (format 4xx-xxx-x)
  No Longer Polymer (NLP) number (format 5xx-xxx-x)
  EC Number appointed under REACH procedure (format 6xx-xxx-x or 7xx-xxx-x)

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.

In 2006, Commission Decision 2006/257/EC established the most recent official list containing the common nomenclature of ingredients employed in cosmetic products [2006/257/EC].

From 11 July 2013 on, the INCI list will be replaced by the so-called "Common Ingredients glossary" [2009/1223/EC]. The new glossary will contain the harmonised names of approximately 20,000 cosmetic ingredients.

4. COSING - EC INFORMATION ON COSMETIC INGREDIENTS

The CosIng (Cosmetic ingredients) database[1] is a publicly available information database in two parts, linked together whenever possible. One part aims at containing all the regulations introduced by the Cosmetic Directive/Regulation. This part contains the historical data since the beginning of the Cosmetics Directive in 1976. The scientific opinions, which are the basis for many of the authorised substances or the restrictions of the substances in the Annexes, are linked to the regulated substances. Each substance is provided with the chemical name, INN name or IUPAC-name, CAS- and EC number, Annex and entry number and the conditions and warnings for its use.

The other part of the database contains the EU-inventory, which is a list of assigned INCI-names to substances offered for sale to the cosmetic industry. In addition to the INCI-name, if possible the CAS- and EC number, chemical name or its description is added, together with the function in the cosmetic products and finally any restrictions imposed by the Cosmetics Directive.

Every possible link between the 2 parts have been established.

5. PART 3 OF ANNEX VI TO REGULATION (EC) NO 1272/2008

Part 3 of Annex VI to Regulation (EC) No 1272/2008 provides the harmonised European classification of a large number of dangerous substances according to the principles laid down in Annex I to that same Regulation [2008/1272/EC]. Annex VI Part 3 previously was Annex I to Directive 67/548/EEC, which was repealed in December 2010. The European harmonised classification Annex is updated on a regular basis and contains a large number of chemicals that can be found in the composition of cosmetic products. It is useful to check the harmonised classification of a compound of interest, but it is of particular importance with regard to **Art. 4b** of the Cosmetic Products Directive, which states [2009/1223/EC]:

The use in cosmetic products of substances classified as carcinogenic, germ cell mutagenic or toxic for reproduction, of category 1A, 1B and 2, under part 3 of Annex VI to Regulation (EC) No 1272/2008 shall be prohibited ... A substance classified in category 2 may be used in cosmetics if the substance has been evaluated by the Scientific Committee on Consumer Safety (SCCS) and found acceptable for use in cosmetic products.

[1] http://ec.europa.eu/consumers/cosmetics/cosing/, consulted December 2010

6. REFERENCES

67/548/EEC - Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.
Official Journal P 196, 16/08/1967 p.1.

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.
Official Journal L 132, 01/06/1996 p.1.

2001/59/EC - Commission Directive 2001/59/EC of 6 August 2001 adapting to technical progress for the 28th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.
Official Journal L 225, 21/08/2001 p.1.

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.

2006/257/EC - Commission Decision of 9 February 2006 amending Decision 96/335/EC establishing an inventory and a common nomenclature of ingredients employed in cosmetic products
Official Journal L97, 05/04/2006 p.1.

2008/1272/EC - Regulation (EC) No 1272/2008 of the European Parliament and the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.
Official Journal L 353, 31/12/2008 p.1.

2009/1223/EC - Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (recast)
Official Journal L 342, 22/12/2009 p.59.

DG24/XXIV/1891/98: Mandate for the SCCNFP Specific Working Group on Inventory, 2 March 1998

SCCNFP/0098/99: Status report on the inventory of cosmetic ingredients, *approved by the plenary session of the SCCNFP on 17 February 1999*.

SCCNFP/0299/00: 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*.

SCCNFP/0389/00: Opinion concerning the 1st update of the inventory of ingredients employed in cosmetic products. Section II: perfume and aromatic raw materials, *adopted by the SCCNFP during the plenary session of 24 October 2000*.

APPENDIX 2: STANDARD FORMAT OF THE OPINIONS

SCCS/xxxx/xx

EUROPEAN COMMISSION

Directorate-General for Health & Consumers

Scientific Committee on Consumer Safety

SCCS

OPINION ON

……..

Scientific Committees

on consumer safety
on emerging and newly identified health risks
on health and environmental risks

The SCCS adopted this opinion at its xxth plenary meeting of xx xxxx 20xx
(by written procedure on date xxxx)

About the Scientific Committees
Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Evaluation Agency (EMEA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS
The Committee shall provide opinions on questions concerning all types of health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example: cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

Scientific Committee members
Jürgen Angerer, Ulrike Bernauer, Claire Chambers, Qasim Chaudhry, Gisela Degen, Thomas Platzek, Suresh Chandra Rastogi, Vera Rogiers, Christophe Rousselle, Tore Sanner, Kai Savolainen, Jacqueline Van Engelen, Maria Pilar Vinardell, Rosemary Waring, Ian R. White

Contact
European Commission
Health & Consumers
Directorate C: Public Health and Risk Assessment
Unit C7 - Risk Assessment
Office: B232 B-1049 Brussels
Sanco-Sc6-Secretariat@ec.europa.eu

(ISSN)

http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

ACKNOWLEDGMENTS

List of members of the concerned working group,
with identification of chair and rapporteur.

……
……
……
……
……
……
……
……
……
……
……
……
……

External experts (if applicable):

……
……
……
……
……
……

Keywords: ……; ……; ……; ……; ……

Opinion to be cited as: ……
……

TABLE OF CONTENTS

ACKNOWLEDGMENTS x

1. BACKGROUND x

2. TERMS OF REFERENCE x

3. OPINION x

4. CONCLUSION x

5. MINORITY OPINION x

6. REFERENCES x

1. BACKGROUND

2. TERMS OF REFERENCE

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Ref.:

3.1.1.2 Chemical names

Ref.:

3.1.1.3 Trade names and abbreviations

Ref.:

3.1.1.4 CAS / EC number

Ref.:

3.1.1.5 Structural formula

Ref.:

3.1.1.6 Empirical formula

Ref.:

3.1.2 Physical form

Ref.:

3.1.3 Molecular weight

Ref.:

3.1.4 Purity, composition and substance codes

Ref.:

3.1.5 Impurities / accompanying contaminants

Ref.:

3.1.6 Solubility

Ref.:

3.1.7 Partition coefficient (Log P_{ow})

Ref.:

3.1.8 Additional physical and chemical specifications

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

Ref.:

3.1.9 Stability

Ref.:

3.2 FUNCTION AND USES

3.3 TOXICOLOGICAL EVALUATION

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

Ref.:

3.3.1.2 Acute dermal toxicity

Ref.:

3.3.1.3 Acute inhalation toxicity

Ref.:

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

Ref.:

3.3.2.2 Mucous membrane irritation

Ref.:

3.3.3 Skin sensitisation

Ref.:

3.3.4 Dermal / percutaneous absorption

Ref.:

3.3.5 Repeated dose toxicity

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

Ref.:

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

Ref.:

3.3.5.3 Chronic (> 12 months) toxicity

Ref.:

3.3.6 Mutagenicity / genotoxicity

3.3.6.1 Mutagenicity / genotoxicity *in vitro*

Ref.:

3.3.8.2 Mutagenicity / genotoxicity *in vivo*

Ref.:

3.3.7 Carcinogenicity

Ref.:

3.3.8 Reproductive toxicity

3.3.8.1 Two generation reproduction toxicity

Ref.:

3.3.8.2 Teratogenicity

Ref.:

3.3.9 Toxicokinetics

Ref.:

3.3.10 Photo-induced toxicity

3.3.10.1 Phototoxicity/photoirritation and photosensitisation

Ref.:

3.3.10.2 Phototoxicity / photomutagenicity / photoclastogenicity

Ref.:

3.3.11 Human data

Ref.:

3.3.12 Special investigations

Ref.:

3.3.13 Safety evaluation (including calculation of the MoS)

Ref.:

3.3.14 Discussion

4. CONCLUSION

5. MINORITY OPINION

6. REFERENCES