Scientific Committee on Consumer Safety

SCCS

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

8TH REVISION

The SCCS adopted this opinion at its 17th plenary meeting
of 11 December 2012

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 substances in Europe. It is designed to provide guidance to public authorities and cosmetic industry, in order to improve harmonised compliance with the actual cosmetic EU legislation. An important development is the 2009 legislative recast which transforms the cosmetic Directive 76/768/EEC[1] into a Regulation. It must be emphasised that from 11 July 2013 onwards this Regulation (2009/1223/EC[2]) is fully applicable. In the meantime Directive 76/768/EEC with the 6th (Directive 93/35/EEC[3]) and 7th (Directive 2003/15/EC[4]) amendments to this Directive still may be applied.

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

The previous revision of the Notes of Guidance took place in 2011 (SCCS/1416/11)[5]. 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 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 in the EU.
Input of scientists from industry, other scientific committees (SCHER, SCENIHR) and Cosmetics Europe (formerly 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 **Regulation (EC) No 1223/2009** of the European Parliament and of the Council of 30 November 2009 on cosmetic products (recast).
Official Journal L342, 22/12/2009 p 59.

3 **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.

4 **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.

5 **SCCS/1416/11**: The SCCS's Notes of Guidance for the testing of cosmetic ingredients and their safety evaluation, 7th revision, *adopted by the SCCS during the 10th plenary meeting of 22 March 2011*.

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ABBREVIATIONS AND GLOSSARY OF TERMS

3R	Refinement, Reduction, Replacement
3T3 NRU PT	3T3 Neutral Red Uptake Phototoxicity Test
Acceptability test	A test intended to confirm the fulfilment of the expectations for a cosmetic product in-use [SCCNFP/0068/98]
ACGIH	American Conference of Governmental Industrial Hygienists
ADME	Absorption, distribution, metabolism, excretion
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]
AOP	Adverse outcome pathway
Art.	Article
ATP	Adenosine Triphosphate
BCOP	Bovine Corneal Opacity and Permeability
BE	Biological Equivalents
BEI	Biological Exposure Indices
BMD	BenchMark Dose 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.
BMDL	BMD Lower limit The BMD lower limit (BMDL) refers to the corresponding lower limits of a one-sided 95% confidence interval on the BMD.
BMR	BenchMark Response
BrdU	5-bromo-2-deoxy-uridine
BSE	Bovine Spongiform Encephalopathy
BW	Body Weight
CAS n°	Chemical Abstracts Service registry number
Cat.	Category
cfu	Colony forming unit
CI	Colour Index
CIN	Common Ingredient Name
CLP	Classification, Labelling and Packaging of Substances and Mixtures
CMR	Carcinogenic, Mutagenic, toxic to Reproduction
Colipa	Cosmetics Europe (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 external 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, protecting them, keeping them in good condition or correcting body odours [2009/1223/EC]
Cosmetics Europe	The Personal Care Association (formerly Colipa)
CPDB	Carcinogen Potency Database
CPSR	Cosmetic Product Safety Report
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 2006]
DG	Directorate-General
DG ENTR	Directorate-General Enterprise
DG ENV	Directorate-General Environment
DG SANCO	Directorate-General Health and Consumer Protection
DHA	Dihydroxyacetone
Dir.	Directive
DNA	DeoxyriboNucleic Acid
Doc.	Document
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.

[1] Used in the calculation of the Systemic Exposure Dosage (see section 3-7.2).

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	"Dose descriptor" is used to designate the exposure level (dose or concentration) that corresponds to a quantified level of risk of a health effect in a specific study [EChA 2008b] As used under chapter 3-7.4 Assessment of carcinogens, it is 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. T25) [Dybing et al. 1997]
DPRA	Direct Peptide Reactivity Assay
EC	European Community
EC Number	EC number, meaning either EINECS number, ELINCS number, NLP number or EC Number appointed under REACH procedure
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
ELISA	Enzyme-Linked Immunosorbent Assay
EMA/EMEA	European Medicines Agency
ESAC	ECVAM Scientific Advisory Committee
EST	Embryonic Stem cell Test
EST-1000	Epidermal Skin Test-1000
EU	European Union
EURL-ECVAM	European Union Reference Laboratory - European Centre for the Validation of Alternative Methods
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 end user, or its prototype [2009/1223/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
GUM	Gesellschaft für Umweltmutationsforschung
Hair product	A cosmetic product which is intended to be applied on the hair of head or face, except eye lashes [2009/1223/EC]
HBM	Human Biomonitoring

HET-CAM	Hen's Egg Test-Chorio Allantoic Membrane
HPRT	Hypoxanthine-guanine PhosphoRibosyl Transferase
HPT-axis	Hypothalamic-pituitary-thyroid-axis
HPV	High Production Volume
HT25	Human dose-descriptor, derived from T25 and based on comparative metabolic rates [Sanner et al. 2001]
IARC	International Agency for Research on Cancer
ICCG	Inter-Committee Coordination Group
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
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 etc. [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
ISO	International Organization for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
JRC	Joint Research Centre
LC_{50}	Median Lethal Concentration 50%: a time dependent, statistically derived estimate of a test article concentration that can be expected to cause death during exposure or within a fixed time after exposure in 50% of animals exposed for a specified time {expressed as mass of test article per unit volume of air (mg/L, mg/m^3) or as a unit volume of test article per unit volume of air (ppm, ppb)} [OECD 2009a].
LCR	Lifetime cancer risk
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	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. [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
Nanomaterial	An insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm. [2009/1223/EC]
NICEATM	The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NLP	No Longer Polymer
NOAEC	No observable adverse effect concentration
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.
NRU	Neutral Red Uptake
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PBPK modelling	Physiologically based pharmacokinetic modelling
PBTK modelling	Physiologically based toxicokinetic modelling
PCPC	Personal Care Products Council (formerly CTFA - Cosmetic, Toiletry and Fragrance Association)
Ph. Eur.	European Pharmacopoeia
PIF	Product Information File
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. [2009/1223/EC]
QSAR	Quantitative Structure-Activity Relationship
RBC	Red Blood Cell
RICC	Relative increase in cell counts
RPD	Relative population doubling
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
SCs	Scientific Committees
SD	Standard Deviation of the mean
SED	The Systemic Exposure Dosage of a cosmetic substance 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 3-7.2: dermal absorption issues).
Serious undesirable effect	An undesirable effect which results in temporary or permanent functional incapacity, disability, hospitalisation, congenital anomalies or an immediate vital risk or death [2009/1223/EC]
SHE	Syrian Hamster Embryo
SI	Stimulation Index
SIT	Skin Irritation Test
SRM	Specified Risk Material
SSA[2]	Skin Surface Area
SSC	Scientific Steering Committee
STE	Short Time Exposure
Substance	A chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition [2009/1223/EC]
Syndet	Synthetic detergent
T25	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 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. [Peto et al. 1984]
TER	Transcutaneous Electrical Resistance
TEWL	TransEpidermal Water Loss
TIF	Technical Information File

[2] used in the calculation of the Systemic Exposure Dosage (see section 3-7.2).

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
UN	United Nations
Undesirable effect	An adverse reaction for human health attributable to the normal or reasonably foreseeable use of a cosmetic product [2009/1223/EC]
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
WoE	Weight of Evidence

* available through http://www.oecd.org/document/55/0,2340,en_2649_34377_2349687_1_1_1_1,00.html, consulted September 2012.

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1. INTRODUCTION

According to Article 2.1 (a) of Regulation (EC) No 1223/2009, *a **cosmetic product** means any **substance** or **mixture** intended to be placed in contact with the external 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, protecting them, keeping them in good condition or correcting body odours.*
"Substance" is defined by Article 2.1 (b) of this Regulation as *a chemical element and its compounds in the natural state or obtained by any manufacturing process, including any additive necessary to preserve its stability and any impurity deriving from the process used but excluding any solvent which may be separated without affecting the stability of the substance or changing its composition.* Whereas Article 2.1 (c) defines *"**mixture"** as a mixture or solution composed of two or more substances.*

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

Article 3 of the Cosmetics Regulation specifies *that a cosmetic product made available on the market shall be safe for human health when used under normal or reasonably foreseeable conditions of use.* 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 and sensitive groups of the population may be involved. Therefore, the safety-in-use of cosmetic products has been established in Europe by controlling the substances, their chemical structures, toxicity profiles, and exposure patterns [2009/1223/EC[1]].

For those substances for which some concern exists with respect to human health (e.g. colourants, preservatives, UV-filters), safety evaluation is done at the Commission level by a scientific committee, presently called Scientific Committee on Consumer Safety (SCCS). These substances are taken up in the Annexes of Regulation (EC) No 1223/2009, replacing Directive 76/768/EEC from 11 July 2013 onwards.

For safety evaluation of cosmetic substances all available scientific data are being considered, including the physical and chemical properties of the compounds under investigation, results obtained from (Q)SAR {(quantitative) structure activity relationship} calculations, chemical categories, grouping, read-across, physiologically based pharmacokinetics (PBPK) /toxicokinetics (PBTK) modelling, *in vitro* experiments and data obtained from animal studies (*in vivo*). Also clinical data, epidemiological studies, information derived from accidents and any other human data are taken into consideration.

With the implementation of Directive 2003/15/EC[2], the need for validated alternative methods, in particular *in vitro* replacement methods, for the safety evaluation of cosmetic substances and products became crucial. This is maintained in Regulation (EC) No 1223/2009.

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1 **Regulation (EC) No 1223/2009** of the European Parliament and of the Council of 30 November 2009 on cosmetic products (recast).
Official Journal L342, 22/12/2009 p 59.

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.

In the present update, the state-of-the-art with respect to the full 3R (Refinement, Reduction and Replacement) strategy of Russell et al [1959[1]], is incorporated.
In particular, the SCCS gives special attention to those alternative methods that are suitable for the safety testing of cosmetic substances. These are taken up in the appropriate chapters.

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], 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.

Although the "Notes of Guidance" are mainly concerned with testing and safety evaluation of the cosmetic substances listed in the Annexes of Regulation (EC) No 1223/2009 and those for which safety concerns have been expressed, they are also of interest to all substances intended to be incorporated in a cosmetic product. Even though 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 actually required by Regulation (EC) No 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 substances and 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 substances.
[ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04/index_en.htm, consulted September 2012]

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, consulted September 2012]

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 substances and products and those related to other non-food consumer products.
Whenever cosmetic substances 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 Substances (individual substance 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

[1] See Article 31 of Regulation (EC) No 1223/2009
[2] http://ec.europa.eu/health/scientific_committees/docs/rules_procedure_en.pdf, consulted September 2012.
[3] http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm, consulted September 2012

which are subsequently considered by the SCCS and incorporated when considered appropriate.

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 substances included in Annexes II, III, IV, VI and VII of Council Directive 76/768/EEC or Annexes II, III, IV, V and VI of the Cosmetic Regulation (EC) No 1223/2009, but also to a broad range of scientific issues related to the safety of cosmetic substances 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 substances. 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,
  7th Revision: SCCS/1416/11.

The Notes of Guidance are regularly updated in order to incorporate new knowledge and scientific advances. Therefore submitted dossiers should be in accordance with the latest published version.

As cosmetic substances are chemical substances, the Notes of Guidance include the toxicological test procedures reported in Commission Regulation (EC) No 440/2008. 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 *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)

[1] http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm, consulted September 2012

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], taken over now by Regulation (EC) No 1223/2009, 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 substances and finished cosmetic products.

2-4.2 Cosmetic substances included in Annexes II, III, IV, V and VI of Regulation (EC) No 1223/2009

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 both the SCCP and the SCCS have added more than 200 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). In the future, opinions will be taken up in the Annexes of Regulation (EC) No 1223/2009. Note that the numbering of these Annexes is slightly different from that of Directive 76/768/EEC:

	REGULATION (EC) NO 1223/2009	**DIRECTIVE 76/768/EEC**
Annex I	Cosmetic Product Safety Report	Illustrative list by category of cosmetic products
Annex II	Prohibited substances	Prohibited substances
Annex III	Restrictions	Restrictions
Annex IV	List of colorants	List of colouring agents
Annex V	Preservatives	List of substances excluded from the scope of the Directive
Annex VI	UV-filters	Preservatives
Annex VII	Symbols used on packaging/container	UV-filters
Annex VIII	List of validated alternative methods to animal testing	Symbols used on packaging/container
Annex IX	**Part A** Repealed Directive with its successive amendments **Part B** List of time-limits for transposition into national law and application	List of validated alternative methods to animal testing
Annex X	Correlation table between Directive 76/768/EEC and Regulation (EC) No 1223/2009	**-**

It should be noted that Regulation (EC) No 1223/2009 defines, for the purpose of the Annexes II to VI a "**hair product**" as *a cosmetic product which is* ***intended to be applied on the hair of head or face, except eye lashes*****.** For other definitions, see Preamble to Annexes II to IV, 2009/1223/EC.

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 substances for inclusion in one of the Annexes of Regulation (EC) No 1223/2009 (previously 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 (non-exhaustive list):

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	SCCNFP/0546/02 SCCP/1111/07 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	SCCNFP/0451/01 SCCNFP/0521/01 SCCNFP/0552/02 SCCNFP/0612/02 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	SCCNFP/0474/01 SCCNFP/0825/04 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	SCCNFP/0484/01 SCCNFP/0797/04 SCCP/0930/05
- safety review of the use of certain azo-dyes	SCCNFP/0495/01
- assessment strategies for hair dyes	SCCNFP/0553/02 SCCP/0959/05
- genotoxicity/mutagenicity testing of hair dyes	SCCNFP/0566/02 SCCNFP/0720/03 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	SCCNFP/0808/04 SCCP/0941/05 SCCP/1004/06 SCCS/1311/10
- hair dyes and skin sensitisation	SCCP 2006 SCCP/1104/07
- hair dye substances and hydrogen peroxide used in products to colour eyelashes	SCCS/1475/12
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	SCCP/0882/05 SCCP/1214/09
- parabens	SCCS/1348/10 SCCS/1446/11
- products resembling food and/or having child appealing properties	SCCS/1359/10
- nitrosamines in balloons	SCCS/1486/12
Fragrance allergy in consumers	
- fragrance allergy in consumers	SCCNFP/0017/98 SCCNFP/0450/01
- prohibited/restricted perfumery materials	SCCNFP/0320/00 SCCNFP/0392/00 SCCNFP/0770/03 SCCNFP/0771/03 SCCNFP/1023/06
- sensitisation quantitative risk assessment (QRA)	SCCP/1153/08
- fragrance allergens in cosmetic products	SCCS/1459/11

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	SCCS/1484/12
- Threshold of Toxicological Concern (TTC)	SCHER/SCCP/ SCENIHR 2009 SCCP/1171/08

3. SAFETY EVALUATION OF COSMETIC SUBSTANCES

3-1 INTRODUCTION

Cosmetics in Europe are regulated by Directive 76/768/EEC and its amendments until 11 July 2013. From then onwards **Regulation (EC) No 1223/2009 applies**, meaning that its provisions will be of application in all Member States, without being subject to potential alterations when translated into national legislations. In principle, the content of Directive 76/768/EEC has been taken over in the Regulation, but certain elements have been strengthened to ensure a high level of protection of human health. Indeed, when a cosmetic product is placed on the EU market it must be safe (Art. 3), one must be in a position to demonstrate the safety (Arts. 10-11) and adequate information must be given to (i) authorities to facilitate in-market control (Art. 13, Notification) and (ii) consumers to ensure safe use (Arts. 19-21).

Safety of cosmetic products is in the EU based on the safety of the ingredients, the latter being evaluated by toxicological testing. Until recently, this was done by using experimental animals. Deadlines for animal testing, however, are imposed and laid down in Directive 2003/15/EC, the so-called 7th Amendment of Directive 76/768/EEC, making **the use of validated alternative methods in toxicological testing compulsory. Only replacement** methods are allowed. Via the combination of a testing and marketing ban, *in vivo* testing outside the EU is only allowed for repeated dose toxicity (including skin sensitisation testing), developmental toxicity and toxicokinetics until 11 March 2013. This is also taken over in Regulation (EC) No 1223/2009 (Art. 18).

The rationale behind the safety of the cosmetic product being based on the safety of its ingredients comes from the fact that many thousands of different cosmetic products on the EU market are all derived from a limited number of substances. 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 substances currently covering colouring agents, preservatives and UV filters (Annexes IV, V and VI to Regulation (EC) No 1223/2009) and banned and restricted substances, respectively (Annexes II and III to Regulation (EC) No 1223/2009).

Other measures to safeguard the consumer health have been taken up in the Cosmetic Regulation. These oblige the responsible person to keep and update a **product information file** (PIF), including the **Cosmetic Product Safety Report** (CPSR) referred to in **article 10 (1)**, whenever a product is placed on the market. Requirements for the PIF are listed in article 11 and the minimum content of the CPSR is listed in **Annex I** of the Regulation. The CPSR consists of two parts: (i) the Cosmetic product safety information and (ii) the Cosmetic product safety assessment, including the name and address of the safety assessor, the proof of qualification of the latter and the date and signature of the safety assessor.

A number of new definitions are introduced in the recast (Art. 2) including "cosmetic product", "substance", "(serious) undesirable effects", "nanomaterials" etc. The important definition of "**responsible person**" is also foreseen (Art. 4), being *a legal or natural person established within the Community* (i.e. the manufacturer, importer or distributor). According to 2009/1223/EC (Art. 4) *only cosmetic products for which a legal or natural person is designated within the Community as "responsible person" shall be placed on the market.* The responsible person shall ensure compliance with the relevant obligations set out in the Cosmetic Regulation.

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

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

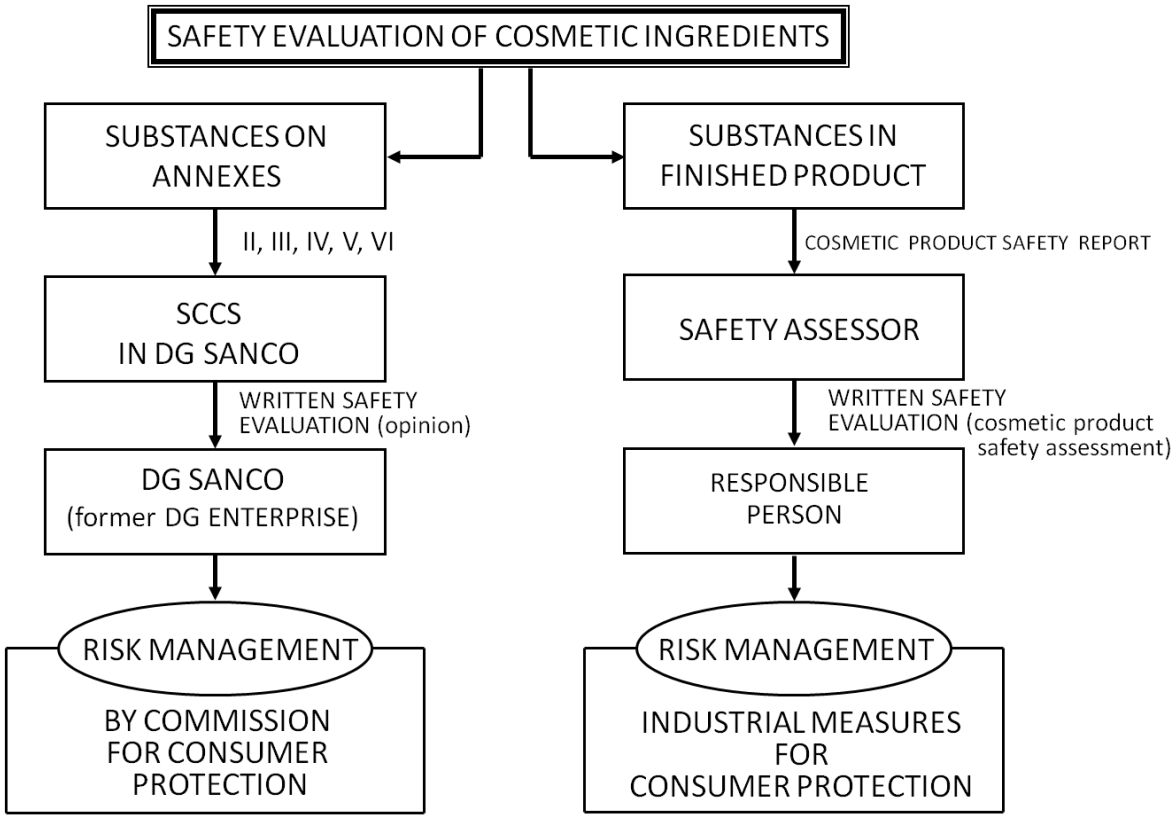

Fig.1: Safety evaluation of cosmetic ingredients in the EU.

It is primarily the substances in Annexes II, III, IV, V and VI that fall under the responsibility of the SCCS. The right part of Fig.1, containing all ingredients of cosmetic products other than the substances present on the Annexes, is the responsibility of the "responsible person", as defined by 2009/1223/EC, through the safety assessor. In general, the **safety evaluation** of cosmetic substances 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, plant protection products, food additives …

This risk assessment procedure is subdivided in 4 parts:

1) **Hazard identification** is carried out to identify whether the substance has the potential to damage human health. It is 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. Also intrinsic physical, chemical and toxicological properties of the molecule under consideration are taken into consideration.

2) **Dose-response assessment:** In this part 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. The Benchmark Dose (BMD) is proposed as an alternative for the 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.
In the case of non-threshold carcinogens, a dose-descriptor (e.g. T25) is determined [Dybing et al. 1997].

3) **Exposure assessment**: In this part 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:** Here 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.

   $$MoS = \frac{NOAEL}{SED}$$ 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. T25) [Dybing et al. 1997]. The assessment of carcinogens is described in Section 3-7.5.

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 substances 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 cosmetic substances presented to the SCCS for consideration, e.g. physical and chemical data, results of relevant toxicity studies, etc.

The contact point for regulatory questions and dossier submissions is:
DG Health and Consumers, Unit B2 Health technology and Cosmetics of the European Commission, SANCO-COSMETICS-AND-MEDICAL-DEVICES@ec.europa.eu

The SCCS address for scientific requests is: SANCO-SCCS-SECRETARIAT@ec.europa.eu

3-3 CHEMICAL AND PHYSICAL SPECIFICATIONS OF COSMETIC SUBSTANCES

Physical and chemical properties of substances 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 substance (e.g. explosiveness, flammability). In addition, some QSAR programmes and empirical models use physical and chemical property values as inputs [Salminen 2002].
The SCCNFP opinion on the basic requirements for toxicological dossiers to be evaluated by the SCCNFP [SCCNFP/0633/02], provides the basic and minimal specifications for any substance to be evaluated. During the years a number of additional points have been added. The actual list is as follows:

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) UV-VIS-absorption spectrum;
11) isomer composition;
12) function and uses.

Original data on all these points must be included in each toxicological dossier and **information and documentation for all analytical data should be provided.** The appropriate **certificate of analysis** must also 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 relevant batches compared to calculated values (e.g. log P_{ow}) or literature data (often different batches are 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 substance under consideration and its structural formula must be given. The Chemical Abstracts Service (CAS) No. of the chemical, the International Nomenclature of Cosmetic Ingredients (INCI) name or Common Ingredient Nomenclature (CIN, as in Reg. 1223/2009) name and the EC number (see Appendix 1 for more detail) should be provided.

With regard to substances 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 substance (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).

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

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 have to be justified.

3-3.2 Physical form

A description of the physical form should be given: powder, paste, gel, liquid… For nanoparticles (see section 3-6.7) the particle size and its distribution should also be given.

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 responsible person must ensure that neither other impurities nor an increase in the impurities are present in the representative commercial material.

3-3.6 Solubility

The solubility [EC A.6] of the substance 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 substance, the UV light absorption spectrum of the compound should be included. It is self-evident that for UV absorbers and UV-filters, this spectrum is absolutely indispensable.
- for nanomaterials and nanoparticles special requirements apply (see also under 3-6.7).

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 UV-VIS-absorption spectrum

Depending on the structure of the substance involved, a UV or UV-VIS-absorption spectrum should be provided.

3-3.11 Isomer composition

When a cosmetic substance is a mixture of isomers, only the relevant isomer used as a cosmetic ingredient should be included in the safety assessment. Information on isomer composition, however, should also be provided.

3-3.12 Functions and uses

For cosmetic substances under study, concentration, function and mode of action (if available) in marketed cosmetic products should be reported. In particular, if cosmetic substances are meant to be included in sprays or aerosols, this should be explicitly mentioned as exposure via inhalation is possible and should be taken into consideration in the risk assessment.
In addition, other uses and the concentrations involved should wherever possible be described (e.g. consumer products, industrial products).

3-4 RELEVANT TOXICITY STUDIES ON COSMETIC SUBSTANCES

The determination of the toxic potential of a cosmetic substance 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 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).
In this respect, some refinement and reduction improvements have been made to existing *in vivo* guidelines. Moreover a number of replacement guidelines, based on *in vitro* methods have been developed. Replacement methods exist 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 and Pauwels 2005, JRC 2010, Adler et al. 2011].
Through the provisions of its 7th Amendment [2003/15/EC], the European cosmetic legislation prohibits the marketing of finished products containing ingredients or combinations of ingredients that have 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 substances. Such valid methods have not necessarily gone through the complete validation process, but the Committee may consider them acceptable when

[1] http://www.unece.org/trans/danger/publi/ghs/ghs_welcome_e.html, consulted September 2012

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]. The imposed deadlines for animal testing and the safety assessment requirements as explained have been taken over in Regulation (EC) No 1223/2009.

This chapter describes the currently used **animal tests and/or their existing validated 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** used in safety assessment, it is essential that **the date of the experiment** is stated. This date not only may explain certain shortcomings in the studies when performed before the existence of the present guideline, 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. In the current chemical 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.
- 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

The original test for acute inhalation toxicity, OECD 403, dates from 1981 and was revised in 2009 in the light of scientific progress, changing regulatory needs and animal welfare considerations [OECD 403/EC B.2]. Furthermore, a reduction and refinement method [EC B.52, OECD 436], describes the **acute toxic class** method by the inhalation route. OECD 433 is a draft guideline of the **fixed concentration procedure** by inhalation [OECD 433].

3) Acute dermal toxicity

No validated alternatives for the *in vivo* acute dermal toxicity assay [EC B.3, OECD 402] are available, but a draft OECD 434 exists for the **fixed dose procedure**.

Usually acute toxicity data of cosmetic substances 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]. Cosmetic products containing substances 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

According to the CLP regulation [2008/1272/EC] from 2011 onwards, skin corrosion is called category 1 and consists of 3 subcategories 1A, 1B, 1C; skin irritation is considered as category 2. It is used for the classification of chemical substances and triggers a number of label elements {(CLP) based on the UN GHS}.

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 substance 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*** **replacement alternatives** have been 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 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 using three to six rabbits. Over the years, the test method has been subject to refinement and reduction measures, bringing the number of animals down to a maximum of three, and now involving a number of steps to be taken before the *in vivo* study can even be envisaged [EC B.4, OECD 404]. 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.

A number of ***in vitro*** **skin irritation tests have been officially validated** [EC B.46]:

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

The performance (specificity and sensitivity) of all three tests has been re-evaluated under the new EU CLP Regulation and was found satisfactory [ESAC 2009a]. Thus the *in vitro* test methods, based on reconstructed human epidermis: EpiSkin™, modified EpiDerm™ and SkinEthic RhE™ are included in OECD 439 and were endorsed by ESAC. The recently published EC B.46 is the counterpart of OECD 439 [EC B.46, Regulation (EC) No 640/2012]. Depending on the regulatory framework and the classification system in use, OECD 439 may be used to determine the skin irritancy of chemicals as a stand-alone replacement test for in vivo skin irritation testing, or as a partial replacement test, within a tiered testing strategy.

Taking the Episkin™ method as an example, the SCCS expressed concerns with regard to potential interference with the **colour formation by reducing substances, hair dyes and colourants** [SCCP/1145/07]. After study of additional data delivered by industry, the SCCS expressed the opinion that the modified Episkin™ method did not provide sufficient proof that the 3-(4,5)-dimethyl-2-thiazolyl-2,5-dimethyl-2H-tetrazolium bromide (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, Cosmetics Europe (formerly 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 applied.

2) Mucous membrane irritation, eye irritation

Eye irritation tests have been developed to assess 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 uses one to three rabbits. Over the years, the original test method [EC B.5, OECD 405] has been subject to refinement and reduction measures, bringing the number of animals down from maximum six to maximum three, recommending the use of anaesthetics to avoid pain an distress and involving a number of steps to be taken before an *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].

While many ingredient dossiers still contain the results of animal studies performed at an earlier time, it forms part of the assays whose performance is affected by the 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, EC B.47] **and the ICE** (Isolated Chicken Eye) [OECD 438, EC B.48]. They can be used in the process of **hazard identification (not risk assessment)** and allow the **elimination of severe eye irritants**, but fail to detect mild irritants. Two other screening tests, namely the IRE (Isolated Rabbit Eye) and HET-CAM (Hen's Egg Test-Chorio Allantoic Membrane), also provide supportive evidence for cosmetic ingredient safety assessment [SCCS/1294/10].
Several tests, including human reconstructed tissue models, are presently under evaluation and validation.

Finally, a number of cytotoxicity / cell function-based assays for water soluble substances (the cytosensor microphysiometer test method; the fluorescein leakage test and the neutral red release, fluorescein leakage and red blood cell haemolysis test) underwent retrospective validation and peer review by ESAC [ESAC 2009b]. These tests, however, are only screening assays and are not suitable for determining the potency of eye irritancy. The Fluorescein Leakage test has been adopted by the OECD and is recommended as part of a tiered testing strategy for regulatory classification and labelling but only for limited types of chemicals (*i.e.* water soluble substances and mixtures) [OECD 460]. For the Cytosensor Microphysiometer [OECD X], which has been validated by ECVAM in 2009, a draft OECD guideline is in progress, which will be based on performance standards to be proposed by an ESAC workgroup. This methodology is in particular used in the USA. Another assay based on cytotoxicity and currently under validation is the Short Time Exposure (STE) test, which uses a rabbit corneal cell line to predict eye irritation. But again, these methods need further critical evaluation before they can be considered full replacement methods for eye irritation.

In the light of the imposed testing ban on cosmetic ingredients, Cosmetics Europe (formerly 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, neither a single *in vitro* assay nor a 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.

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

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

1) The **Local Lymph Node Assay (LLNA)** [OECD 429] has been taken up in Regulation (EC) No 440/2008 (Test Methods) [EC B.42] and was recently amended [Regulation (EC) No 640/2012]. This is a **reduction/refinement alternative test** compared to the traditional two *in vivo* tests mentioned below (points 2 and 3). As more than one exposure is necessary, the test could be seen as a "repeated dose" toxicity test [SCCS/1294/10] and therefore the final testing and marketing ban of 11 March 2013 applies. The LLNA 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 a 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].
   Work at the OECD level took place to accept the LLNA using a **non-radioactive methodology**. Both methods have been adopted by the European Commission:
   - Daicel-ATP, which is a modified LLNA method using adenosine triphosphate (ATP) as an endpoint. The mice are exposed 4 times instead of 3 times and the ATP content is used as a measure of the proliferation of the lymph node cells [EC B.50, OECD 442A].
   - Cell proliferation ELISA (Enzyme-Linked Immunosorbent Assay) BrdU (5-bromo-2-deoxy-uridine), which is a 2nd generation ELISA with calorimetric or chemiluminescent detection which quantifies the DNA synthesis within the

lymph node cells (NICEATM-ICCVAM website, test method evaluation report LLNA: BrdU-ELISA[1]) [EC B.51, OECD 442B].

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 [Vandebriel and van Loveren 2010].

Currently, a number of *in vitro* tests, that are representative for the different steps of skin sensitisation are either being developed or in the phase of validation. It is an example of the so-called "Adverse Outcome Pathway" (AOP) approach, which is mechanistic-based and considered to be the way forward in the field of alternatives.

A test to assess peptide reactivity, being the first step in the AOP, namely the Direct Peptide Reactivity Assay (DPRA), has passed ESAC and has been forwarded to OECD. This method measures the ability of chemicals to react with proteins (haptenation), a determinant step in the induction of skin sensitisation. It is based on the chemical reactivity of the compound under investigation, with lysine and cysteine residues [Gerberick et al. 2004].
Another method that has passed ESAC, namely KeratinoSens, measures direct reactivity of sensitising material to key cysteine residues of Keap1, a regulator of Nrf2. The Nrf2-Keap1-ARE regulatory pathway is considered one of the most relevant pathways for the identification of potential skin sensitizers [Natsch 2010].
Both methods can be used in a Weight of Evidence (WoE) approach in an *in vitro* test battery for the assessment of skin sensitisation.

A human cell line activation test (h-CLAT), based on the enhancement of CD86 and/or CD54 expression in THP-1 cells, is in the prevalidation stage at EURL-ECVAM. An extensive review of the actual status of *in vitro* testing in this field can be found in a JRC report [Adler et al. 2011].

3-4.4 Dermal / percutaneous absorption

a. Major guidelines for dermal / percutaneous absorption

Human exposure to cosmetic substances 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 (which depends on the body site), the duration of exposure, the amount of topically applied product,

[1] http://iccvam.niehs.nih.gov/methods/immunotox/llna-ELISA/TMER.htm, consulted September 2012

the concentration of target compounds, occlusion, etc. [for basic reviews see Schaefer et al. 1996; ECETOC 1993; Howes et al. 1996].

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 substances 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 Guidances [DG SANCO 2004, OECD 2004] address 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 *in vitro* dermal / percutaneous absorption studies**.

b. The SCCS "Basic criteria"

The purpose of *in vitro* dermal absorption studies of cosmetic substances is to obtain qualitative and/or quantitative information on the compounds 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 tested.
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.
14) When dermal absorption studies are performed, often radioactive labelling of the substance under consideration is used in order to increase measuring sensitivity. Justification should be given for the type and site of labelling chosen e.g. present or not in ring structure(s) or side chain(s), use of single or double labelling, etc. This information is important with respect to the biotransformation and stability of the compound during the in vitro dermal absorption test.
15) The technical ability of the performing laboratory and the validity of the method used should be assessed at regular intervals, at least twice per year, by using reference compounds like caffeine or benzoic acid. These data should be included in the study report [OECD 2004, Van de Sandt et al. 2004].

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. In the case of substances with very low dermal absorption and limited permeation (e.g. colorants with high molecular weight and low solubility) the epidermis may be excluded when it is demonstrated that no movement of the chemicals from the skin reservoir to the receptor fluid occurs [Yourick et al. 2004, WHO 2006].

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, or no dermal absorption data is available, 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].

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

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 good 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, a default assessment factor of 3 to extrapolate from subacute (28 days) to subchronic (90 days) toxicity 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 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, Adler et al. 2011].

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 substances 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.

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 and the Cosmetic Regulation (EC) No 1223/2009 only allow up to 11 March 2013 the use of *in vivo* tests for repeated exposure.

3-4.6 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 "Reproduction/Developmental Toxicity Screening Test" [OECD 421], as well as a "Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test" [OECD 422], which to date have not been taken up in Regulation (EC) No 440/2008 [2008/440/EC]. However, according to the REACH Regulation (EC) No 1907/2006 [2006/1907/EC], one of these screening tests is mandatory for substances manufactured or imported in quantities of 10 tonnes or more if there is no evidence from available information that the substance may be a developmental toxicant.
Recently, the **Extended One-Generation Reproductive Toxicity Study** has been taken up by the OECD [OECD 443].

A two-generation reproduction toxicity test is often not provided for cosmetic substances. On a case-by-case basis, it could be necessary to run such a test.

Since the field of reproductive toxicity is very complex, it is expected that the various stages cannot be mimicked using one alternative method and that a battery of tests is needed. **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 a substance 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. Therefore *in vivo* rodent studies will remain necessary. The **EST can be considered as a screening test** and further research is required.

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

To this respect, it can be mentioned 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 [Schenk et al. 2010].

An extensive review of the actual situation with respect to *in vitro* testing in this field can be found in a JRC report [Adler et al. 2011].

[1] http://www.reprotect.eu/, consulted September 2012

3-4.7 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 causes 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, 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 that 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 evaluation of the potential for mutagenicity of a cosmetic substance to be included in the Annexes of Regulation (EC) No 1223/2009 should include tests to provide information on the following genetic endpoints, namely 1) mutagenicity at a gene level, 2) chromosome breakage and/or rearrangements (clastogenicity), and 3) numerical chromosome aberrations (aneugenicity). This recommendation represents the actual consensus of international groups of scientific experts [Muller et al. 2003, Dearfield et al. 2011, 2006/1907/EC, EFSA 2011, Kacew and Lee 2013], and of an expert advisory committee [COM 2011]. 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 measure primary DNA damage (instead of irreversible DNA damage) without taking into account the consequences of the damage, should not be used. Moreover, the SCCS recommends before undertaking any testing, a thorough review of all available (literature) data on the substance under study, including its (physical) chemistry, toxicokinetic and toxicological profile, as well as data on analogous substances.

In principle, the **SCCS recommends** for the base level testing of cosmetic substances, **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** [EC B.49, OECD 487]
     or
   - ii) ***In vitro* Mammalian Chromosome Aberration Test** [EC B.10, OECD 473]

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**

[1] http://www.oecd-ilibrary.org, consulted September 2012

number of tests, the number of unexpected positives increases whereas the number of unexpected negatives decreases.

Recently, Kirkland et al. [2011] showed 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 exist in these databases. The combination of these two assays would cover the three endpoints, as the *in vitro* micronucleus assay detects both structural and numerical chromosome aberrations. The European Food Safety Authority (EFSA) has already published an opinion in which the use of 2 tests (OECD 471 and OECD 487) is recommended as a first step in genotoxicity testing for food and feed safety assessment [EFSA 2011]. The guidance of the UK Committee on Mutagenicity also recommends the two tests (Ames and micronucleus test) for stage 1 *in vitro* testing [COM 2011]. Actually, the SCCS is setting up a workshop to discuss the scientific basis of changing the "standard" battery.

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 e.g. nanomaterials or substances with specific structural alerts, it is established that specific protocol modifications/additional tests are necessary for optimal detection of genotoxicity.

In the case of nanoparticles, the bacterial reverse mutation test is not a reliable test. *Salmonella* and *Eschericia* bacteria lack the mechanisms (e.g. endocytosis) to incorporate particles. For nanoparticles a gene mutation test in mammalian cells (*hprt* test, mouse lymphoma assay) is an accepted alternative for the bacterial test.

Although most tests will give clearly positive or clearly negative results, in some cases the outcome has to be considered inconclusive or equivocal. Equivocal refers to a situation where some but not all the requirements for a clear positive or clear negative result have been met. A substance giving an equivocal test result should be reinvestigated, using the same test method, but varying the conditions (including sampling more cells) to obtain conclusive results. Inconclusive refers to a situation where no clear result was achieved due to limitation of the test or procedure. In this case, repeating the test under the correct conditions should produce a clear result.

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 order to demonstrate that the result obtained is due to treatment with the substance, it is essential to demonstrate exposure of the bacteria or cells. A way to demonstrate exposure is through cytotoxicity. In the Ames test a reduction in the number of spontaneous revertant colonies and/or clearing of the bacterial background lawn is sufficient to indicate cytotoxicity and thus exposure of the substance. The other tests, measuring the induction of micronuclei or gene mutations (nanoparticles) in mammalian cells, require that the cells divide through at least (and sometimes at most) one round of replication to convert DNA damage into the genetic endpoint scored by the test. Therefore, cytotoxicity measures based on cell proliferation are preferred and, consequently, have been incorporated into the revised OECD Test Guidelines.

In the *in vitro* micronucleus test, Fowler *et al.* [2012] have shown that the use of relative population doubling (RPD) or relative increase in cell counts (RICC) helps to increase the specificity of the *in vitro* micronucleus test. If cytochalasin B is used to obtain binuclear cells, determination of the reduction in the number of binuclear cells is a justified alternative way to measure cytotoxicity. In gene mutation tests (nanoparticles), relative total growth or relative survival (relative cloning efficiency) is the preferred measure of cytotoxicity.

In cases where negative results are seen in the conducted tests, a mutagenic potential is excluded. Likewise, in cases where a 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]. A number of promising alternative methods are under development. In the future these tests could add to a weight of evidence approach. Examples are:

- the Micronucleus test in reconstructed human skin
- the Comet assay in reconstructed human skin

Also worldwide research is ongoing to overcome false positive *in vitro* results by incorporating *in vitro* toxicogenomics. The idea is that by global gene expression profiling via genome-wide transcriptomics (microarray) technology, gene patterns covering diverse mechanisms of compound-induced genotoxicity can be extracted. These gene patterns/biomarkers can be further used as a follow-up of positive findings of the standard *in vitro* mutagenicity/genotoxicity testing battery [Goodsaid et al. 2010, Doktorova et al. 2012a, Magkoufopoulou et al. 2012].

3-4.8 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]. Carcinogens can be classified into genotoxic carcinogens or non-genotoxic carcinogens. Genotoxic carcinogens induce cancer through interaction with DNA and induction of mutations. In contrast, non-genotoxic carcinogens induce tumors through mechanisms other than DNA damage, *e.g.* hormonal effects. Before the testing/marketing ban of the 7th Amendment of the Cosmetics Directive [2003/15/EC] on cosmetic ingredients, the **most commonly performed carcinogenicity tests** were the Carcinogenicity test [EC B.32, OECD 451] or the Combined chronic toxicity / carcinogenicity test [EC B.33, OECD 453]. Under this testing and marketing ban, *in vivo* testing to investigate the carcinogenic potential of substances is no longer possible. Unfortunately, at present no validated methods to study carcinogenicity are available.

The ***in vitro* Cell Transformation Assay (CTA)** is at a late stage of development. An ESAC workgroup reviewed the prevalidation data available and came to the conclusion that the CTAs using Syrian hamster embryo (SHE) cells tests are promising but need to come to a common protocol. For the CTA using BALB/c 3T3 more work is needed [ESAC 2011]. Recently, all information on the CTA has been taken up in a review booklet [Josephy et al (eds.) 2012]. Also a Detailed Review Paper [OECD 2007] has been published whereas an OECD test guideline is under development.

The advantage of the CTA is that it is assumed to detect both genotoxic and non-genotoxic carcinogens. Indeed, exposure of cultured cells to both types of carcinogenic substances in a CTA test can lead to cell transformation involving changes in cell behaviour/phenotype. Transformed cells can lead to tumour formation *in vivo* when injected in a suitable host, underlining their biological relevance.
Discussions are ongoing with respect to the use of a CTA (i) as a stand-alone test for non-genotoxic carcinogens; (ii) to de-risk a positive *in vitro* genotoxicity test in a WoE

approach (as additional information); (iii) in combination with a positive *in vitro* genotoxicity test to identify a genotoxic carcinogen.

Without the 2-year bioassay, it is very difficult if not impossible to conclude on the carcinogenicity of substances. As far as genotoxic compounds are concerned, *in vitro* mutagenicity tests are quite well developed. Due to the relation between mutations and cancer, these genotoxicity tests can be seen as a pre-screen for cancer. A positive result in one of the genotoxicity tests may be indicative enough to consider a compound as putatively carcinogenic. In combination with the CTA, this indication may even be stronger. However, as carcinogenicity is a multi hit/multi step process, it can (for the time being) not be mimicked by *in vitro* tests. Today, any reliable, justified conclusion on the carcinogenicity of a substance needs *in vivo* tests.

The situation is different for the non-genotoxic carcinogens. Before the animal testing and marketing ban, non-genotoxic carcinogens were detected by the (sub-)chronic repeated dose studies, including the carcinogenicity test. Alternatives for these *in vivo* tests to detect non-genotoxic carcinogens, however, are not available with the exception of the CTA. Therefore, currently, *in vivo* rodent studies are essential to detect non-genotoxic substances.

An extensive review of the actual status of *in vitro* carcinogenicity testing can be found in a JRC report [Adler et al. 2011].

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].

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, 4-methyl benzylidene camphor and n-butylparaben, to address specific questions with respect to human safety [SCCP/0989/06, SCCP/1184/08, SCCS/1348/10, SCCS/1443/11, SCCS/1446/11]. For active ingredients, toxicokinetic data are at the basis of risk assessment e.g. pharmaceuticals, plant protection products. It would be a step forward to include more toxicokinetic studies for Annex cosmetic ingredients.

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 and represent a key tool in an integrated testing strategy, a lot of efforts will be needed to make substantial progress in this field [Coecke et al. 2012]. *In vivo* studies remain necessary.

An extensive review of the actual status in this field can be found in a JRC report [Adler et al. 2011].

3-4.10 Photo-induced toxicity

1) Phototoxicity (photoirritation) and photosensitisation

The "3T3 Neutral Red Uptake Phototoxicity Test (3T3 NRU PT)" is **a validated *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 substances to be used as UV filters [SCCNFP/0069/98].

In 2000, the 3T3 NRU PT test was formally validated and is now taken up in 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 substances. 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 **validated *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].

In certain cases, the validated 3T3 NRU PT test may provide false positive results. It seems quite common practice to further evaluate then as a second tier, the biological effects on a reconstructed human skin model having some barrier properties while checking carefully for the solvents used [Kandarova, 2011].

Recently, a postvalidation exercise of the 3T3 NRU PT took place since in particular for pharmaceutical substances false positives were observed. Some measures (e.g. limit of 100µg/ml as highest concentration) were taken to decrease this number [ECVAM/EFPIA workshop report 2011].

2) Photomutagenicity / Photoclastogenicity

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

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 no results are yet 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 discussion paper by EMA (formerly 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 placed in contact with the external parts of the human body or with the teeth and the mucous membranes of the oral cavity 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 substances, based on animal testing and/or the use of alternative methods, are available and no concern is raised. A high degree of safety needs to be ensured. 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 substances [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) substances [SCCNFP/0120/99]. These types of tests are much more controversial than the irritancy tests, since predictive human sensitisation tests carry the risk to induce a long lasting or permanent immunological 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 immunological 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 REGULATION (EC) NO 1223/2009 (TO BE EVALUATED BY THE SCCS)

3-5.1 General toxicological requirements

When a dossier of a cosmetic substance 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 (skin and eye);*
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 substances 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 substance is present in a cosmetic product is expected or intended to being used on sunlight-exposed skin. 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 undertaking of animal studies, not only the imposed testing and marketing bans in the cosmetic legislation need to be kept in mind, but equally 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 developed in **Art.4** of Directive 2010/63/EU on the protection of animals used for scientific purposes, which introduces the 3R-principle into the European legislation [2010/63/EU].

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;
  - 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 Regulation (EC) No 1223/2009 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 substance in this Annex, unless it belongs to the category of hair dyes or hair dye components (see 3-8).

3-5.4 Annex IV

Annex IV constitutes a list of colouring agents permitted for use in cosmetic products.
A number of these colourants have a wide use in food and have been declared as safe for use for many years, while on others clear restrictions have been imposed.
The data requirements for colourants do not differ from those defined in 3-5.1, unless they are being used as hair dyes or hair dye components (see 3-8).

3-5.5 Annex V

Annex V 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 VI

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

By their nature, all cosmetic substances 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 substance 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 a substance in Annex VI.

Finally, it must be emphasised that all the studies relating to the phototoxic potential of a substance must be performed by applying the relevant UV light wavelengths derived from the absorption spectrum of the substance [SCCNFP/0633/02], and that photostability data under conditions of use should be provided. The absorption spectrum should be added to the data submitted.

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 SUBSTANCES PRESENT IN FINISHED COSMETIC PRODUCTS (WHICH ARE TO BE 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 cosmetic substances taken up in the Annexes to Regulation (EC) No 1223/2009 (previously Directive 76/768/EEC), some general considerations apply to all other potential ingredients of cosmetic products.

Since cosmetic substances 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 substances, however, does not trigger any additional toxicological data requirement under the chemicals legislation.

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 substances 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 substances taken up in the Annexes to Regulation (EC) No 1223/2009, 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 substances 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 issues, caused by the nature and/or origin of the cosmetic substances under consideration, are discussed.

3-6.2 Identification of mineral, animal, botanical and biotechnological ingredients in a cosmetic product

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

a) Complex substances 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 substances 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 substances 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 substances derived from biotechnology

For special biotechnologically derived substances, 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 substances 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/CIN name if available;
- for natural substances, there should be either
  1) an analysis of the composition of the batch of the natural substance, or
  2) an indication of the maximum levels of components which may be present in the natural substance, taking into account batch to batch variation;
- an indication of the substances 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, SCCS/1459/11]; 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 substances 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 substances in Annex III to Regulation (EC) No 1223/2009. More specifically, the presence of these substances must be indicated in the list of substances 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].

The SCCS has adopted an opinion on fragrance allergens in cosmetic products which enlarges the list of fragrance allergens considered relevant for consumers and which allows to derive a general threshold for substances with a higher number of recorded cases [SCCS/1459/11].

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

> *"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[2].
Evidence for a hormone-like activity in screening assays would already categorize a substance as a **potential ED** (endocrine active substance) 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 oestrogens 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[3].

[1] http://ec.europa.eu/environment/endocrine/definitions/endodis_en.htm, consulted September 2012
[2] http://www.oecd.org/document/62/0,2340,en_2649_34377_2348606_1_1_1_1,00.html, consulted September 2012
[3] http://ec.europa.eu/environment/endocrine/definitions/affect_en.htm, consulted September 2012

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[1].

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[2].

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 joint Germany-United Kingdom position paper[3] proposed a regulatory definition of an endocrine disruptor in relation to potential threat to human health. Further discussions are going on as DG Environment set up an "Expert Group on endocrine disruptors" to discuss the scientific criteria to identify endocrine disruptors. In the near future EFSA together with the different scientific committees will take up the mandate with respect to EDs.

Recently two *in vitro* test methods were adopted by OECD [OECD 455, OECD 457] to detect oestrogen receptor antagonists and/or agonists.

Potential endocrine disruptors as cosmetic substances:

In 2001, substances in cosmetic products were first discussed 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 EDs present in cosmetics have been reviewed by SCCP and SCCS, *i.e.* parabens [SCCP/1017/06, SCCP/1183/08, SCCS/1348/10, SCCS/1446/11], 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.

[1] http://ec.europa.eu/environment/endocrine/strategy/short_en.htm, consulted September 2012
[2] http://ec.europa.eu/environment/endocrine/strategy/substances_en.htm, consulted September 2012
[3] http://www.bfr.bund.de/cm/343/regulatory_definition_of_an_endocrine_disrupter_in_relation_to_potential_threat_to_human_health.pdf, consulted September 2012

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 substances 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, Adler et al. 2011].

3-6.5 Animal-derived cosmetic substances, 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 substances 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:

"**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 substances 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 substances 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 substances 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 substance [SCCP/0933/05].

3-6.6 CMR-substances

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 substance, 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 [2008/1272/EC]. 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 into the Cosmetics Regulation makes use of the new nomenclature as stated in Table 1. It foresees that CMR 1A, 1B and 2 are prohibited for use in cosmetics, unless the specific criteria set in Article 15 of the Recast are fulfilled. CMR 2 substances may be used in cosmetics where they have been evaluated by the SCCS and found safe. CMR Cat. 1A or 1B substances may be used in cosmetics by way of exception where (1) they comply with the European food safety requirements[2], (2) they cannot be replaced by suitable alternatives, (3) the application is made for a particular use of the product category with a known exposure and (4) the substances were evaluated and found safe by the SCCS for use in cosmetic products, in particular in view of exposure to these products and taking into consideration the overall exposure from other sources, taking particular account of vulnerable population subgroups [2009/1223/EC]. These substances could be allowed to be used as cosmetic substances within Europe under specific conditions.

Guidance is currently being developed with the aim of enabling a harmonized approach to the development and use of overall exposure estimates in assessing the safe use of CMR substances.

1 http://www.unece.org/trans/danger/publi/ghs/ghs_welcome_e.html, consulted September 2012

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 owing to possible developmental toxic effects*	**Reproductive toxicant Cat. 2** *Suspected human reproductive toxicant*
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

The EU Cosmetics Regulation (Regulation (EC) No 1223/2009) specifically covers the use of nanomaterials in cosmetic products. The Regulation provides a definition of nanomaterial, as well as a mechanism for notification, labeling, and safety evaluation of cosmetic products containing nanomaterials. In this Regulation (EC) No 1223/2009, Article 2 (1) (k), "nanomaterials" means *an insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm*.

The Regulation therefore intends to cover mainly those nanomaterials that are intentionally made, and are insoluble/partially-soluble or biopersistent (e.g. metals, metal oxides, carbon materials, etc), and not those that are soluble or degradable/non-persistent in biological systems (e.g. liposomes, emulsions, etc).

There are other pieces of EU legislation and technical guidance supporting implementation of legislation, with specific references to nanomaterials. To ensure conformity across legislative areas, where often the same materials are used in different contexts, the Commission adopted a Recommendation in 2011 on an overarching definition of a nanomaterial [1]. According to this Recommendation [2011/696/EU] a "Nanomaterial" means:

A natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm – 100 nm [2].

Detailed and technical information about the definition of a nanomaterial is available in the "questions and answers" section[3]. This Recommendation has not yet been applied to the definition of a nanomaterial under the Cosmetic Regulation (EC) No 1223/2009.

In relation to risk assessment, SCENIHR adopted an opinion on the appropriateness of the current methodology in accordance with the technical guidance documents for new and existing substances for assessing the risks of nanomaterial [SCENIHR, 2007] and a document on risk assessment of products of nanotechnologies [SCENIHR, 2009]. 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 2009b]:

- Because of high surface energies, nanoparticles tend **to stick together** to form larger agglomerates and aggregates, or bind with other moieties. However, this can be modified by adding stabilising/dispersing agents. Therefore, the composition of a test medium may lead to substantial **changes in the degree of aggregation/agglomeration** of nanoparticles during the test, and may affect the results. Characterisation of nanoparticles, prior to and during a test, is therefore key to ensuring that valid results are obtained.
- Most test methods were developed and are suitable for substances that are soluble. Insoluble and/or poorly soluble nanoparticles are considered to 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 also requires **ascertaining the stability** of a

[1] http://ec.europa.eu/environment/chemicals/nanotech/pdf/commission_recommendation.pdf, consulted September 2012

[2] In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50% may be replaced by a threshold between 1 and 50%. By derogation, fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials.

[3] http://ec.europa.eu/environment/chemicals/nanotech/questions_answers.htm, consulted September 2012

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 alone may not be appropriate** for nanoparticles. 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, surface area etc.
- Due to the insoluble particulate nature and nano-dimensions, nanoparticles are likely to have an altered uptake and biokinetic profile in a biological system compared to the equivalent conventional forms. The potential 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. **For hazard identification, emphasis should therefore be on toxicological tests over prolonged periods with repeated doses that are followed up by histopathological investigations.**

In view of the special considerations for nanomaterials, the SCCP published an Opinion on Safety of Nanomaterials in Cosmetic Products in 2007 [SCCP/1147/07]. The issues have since been discussed further by the SCCS with a focus on **the safety assessment of nanomaterials in cosmetic products** and a guidance document has recently been issued [SCCS/1484/12]. This is not only meant to facilitate the submission of safety dossiers, but also to assist in the implementation of the provisions of Article 16 of the EU Cosmetic Regulation (EC) No 1223/2009 [2009/1223/EC] which foresees that cosmetics containing nanomaterials will **need to be notified to the Commission 6 months prior to placing on the market.** Some **specific information** as provided in SCCS/1484/12 including material identification, specification, quantity, toxicological profile, safety data and exposure, needs to be provided for any nano-sized cosmetic ingredient used in a cosmetic product. An exception applies for nanomaterials used as colorants, UV-filters or preservatives regulated under Article 14, as their inclusion in the Annexes is in any case subject to a SCCS safety assessment. The notification of cosmetic products containing nanomaterial becomes mandatory from 11 January 2013 onwards. In case the Commission has concerns regarding the safety of a nanomaterial, an **SCCS opinion** shall be sought. In this regard, the following key considerations have been emphasized in the Guidance Document [SCCS/1484/12]:

- For any new or already approved cosmetic ingredient fulfilling the criteria for defining a nanomaterial set up in the Cosmetic Regulation (EC) No 1223/2009, Article 2 (1) (k), as amended, safety data with special considerations to the nano-scale properties will be required for risk assessment.
- Irrespective of the presence of nanomaterials, the existing regulations and SCCS Guidance on Testing of Cosmetic Ingredients and their Safety Evaluation must be followed.
- Detailed characterisation data must be provided on the identity and composition, relating to the same (or justifiably comparable) nanomaterial that is intended for use in the final product. The information should correspond to Cosmetics Regulation (EC) No 1223/2009, Article 16 (3) a) "identification of the nanomaterial…"). The characterisation must also include measurement of

important physico-chemical parameters listed in the SCCS Guidance on the Safety Assessment of Nanomaterials in Cosmetics [SCCS/1484/12], corresponding to Cosmetics Regulation (EC) No 1223/2009, Article 16 (3) b) "specification of the nanomaterial..."

- The characterisation needs to be carried out on the nanomaterial at the raw material stage, in the cosmetic formulation, and during exposure for toxicological evaluations. Where needed, the SCCS may ask further information regarding the description of production processes, any surface modifications, and the preparatory steps carried out for integrating the nanomaterials in the final cosmetic products to facilitate risk assessment.
- The method for calculating dermal and oral exposure to nanomaterials will not be very different from that of conventional cosmetic ingredients, as provided here in the actual document. However, certain assumptions used for estimation of dermal absorption of conventional chemical ingredients are not applicable to nanomaterials. **Dermal absorption of nanomaterials will therefore need to be determined experimentally**.
- For spray application of products containing nanomaterials, **droplet size as well as the size distribution of the dried residual aerosol particles will need to be measured**.
- The likelihood and extent of translocation of nanomaterials across skin, lung, or gastrointestinal barriers (as appropriate) should be determined whilst mimicking the actual use scenarios, with due considerations to nano-aspects.
- Where there is evidence for systemic absorption, further investigations will be required to confirm whether the absorbed material was in a particle form or in solubilised/metabolised form. Where the absorption of particles cannot be excluded either by experimental data, or justified on the basis of solubility/degradation of the nanomaterial, the SCCS may apply a default approach and assume that **100% of the absorbed material was in particle form**.
- Where application of a nanomaterial-containing cosmetic product can lead to systemic exposure, data on toxicological evaluation will be required. Any testing of nanomaterials for hazard identification/ dose response characterisation must be carried out in consideration of the nano-related aspects. Information on the possible local effects will also be required.
- Initial focus of testing should be on ADME (absorption, distribution, metabolism and excretion) parameters to investigate the fate and behaviour of the nanomaterial in the body (*in vivo* or *ex vivo*) and to identify the likely target organs.
- Like other cosmetic ingredients, data on a base set of toxicological endpoints will be required. These include **dermal/ percutaneous absorption, acute toxicity; irritation (skin and eye) and corrosivity, skin sensitisation, repeated dose toxicity, and mutagenicity/ genotoxicity**. Depending on the outcome of the tests, further information **on carcinogenicity, reproductive toxicity** may also be required. Photo-induced toxicity data are specifically required when the cosmetic product is expected or intended for use on sunlight-exposed skin and is able to absorb light.
- At present, the available alternative methods that can be used in place of animal tests are only validated for conventional substances, and not for nanomaterials. The available validated *in vitro* tests may, however, be relevant for hazard identification, in terms of providing additional supporting evidence to the results of *in vivo* studies, and additional information on the possible mechanism(s) of toxic action of nanomaterials, provided that they are carried out with due consideration of the nano-related aspects.
- With respect to the MoS calculation, risk assessment of a nanomaterial might not be different from other conventional ingredients. Where data have been

derived from validated tests, or from relevant and justified tests, and uncertainties are not high, there may not be a scientific reason for applying higher margins of safety to a nanomaterial than is done for a conventional material. However, where this is not the case, and insufficient data, or data from inadequate tests, have been provided, the risk assessor may consider applying additional uncertainty factors for a nanomaterial.

One major obstacle regarding the safety assessment of nanomaterials in cosmetic products is that currently there are limitations in the validity of some of the *in vitro* tests used as they were developed for conventional (soluble) chemicals and not for nanoparticles. For example, in genotoxicity testing, considerations in regard to the exposure of the cells and/or target organs investigated also need to be taken into account.

In the SCCS Guidance document on the Safety Assessment of Nanomaterials in Cosmetics [SCCS/1484/12] more detailed considerations which should be taken into account when assessing the safety of nanomaterials in cosmetic products, can be found.

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

3-7.1 Calculation of the Margin of Safety of a cosmetic substance

In risk characterisation, the last phase in the safety evaluation of a cosmetic substance, 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 substance 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 conclude that a substance is safe for use**.

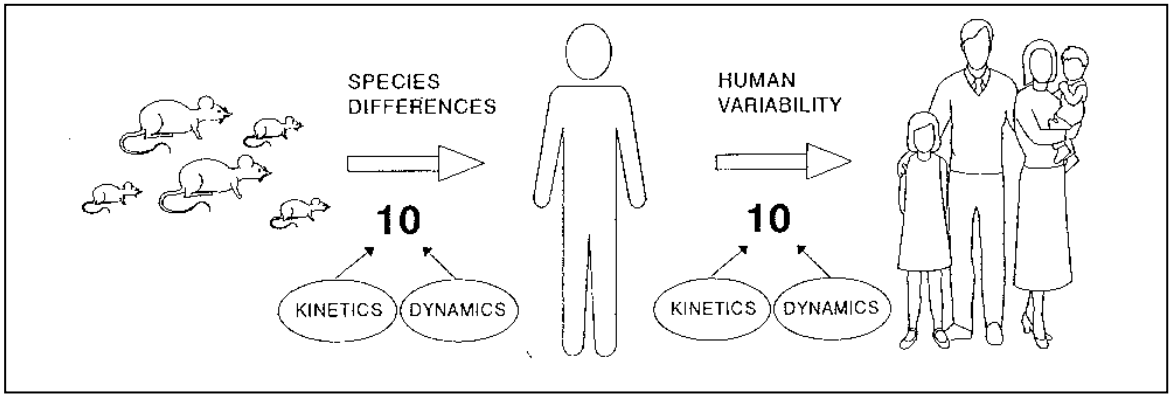

Fig.2: Schematic representation of the extrapolation from animal to man [Renwick, 1998].

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. When considerable qualitative/quantitative kinetic differences are observed in individuals for example it may be necessary to include an additional safety factor (case-by-case evaluation).

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.

In a number of well documented cases, it is possible that the interspecies default factor (factor of 4, see Fig. 3) can be reduced, e.g.:

- presence of relevant pharmacokinetic data for rat and/or human [SCCS/1443/11, SCCS/1479/12]

- presence of different susceptibility to hypothalamic-pituitary-thyroid (HPT)-axis disturbances in rats and humans [SCCS/1481/12]

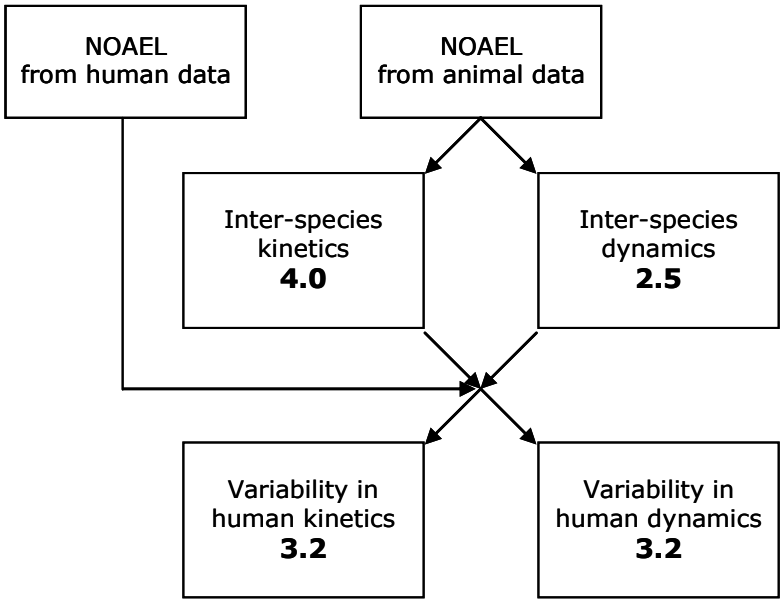

Fig.3: Further subdivision of the Margin of Safety, taking into account kinetics and dynamics [based on WHO, 1994].

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

human body weight value. As such, the amount of substance 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. Then the factor of 50% is applied. 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].

It can be helpful to apply an **existing alternative method to estimate oral absorption** or a combination of some of the existing methods, including QSAR-like models*, in vitro* monolayer cultures (e.g. Caco-2 cells, TC7 cells), artificial membranes (PAMPA), Ussing chamber setup, everted (gut) sac setup and other 3R alternative models [Adler et al. 2011]. Although not officially recognized as a validated alternative method, **Caco-2 cells**, derived from human colon carcinoma, have been **most widely proposed as representing a suitable cell culture model for permeability screening.** Seen the high number of variables involved in the complex process of intestinal absorption [Turco et al. 2011], it is of key importance to work under **well-documented and standardized conditions** in order to be able to draw valid conclusions when such *in vitro* models are being applied [SCCS Expert Methodologies meeting, 2011]. It is therefore necessary to report on all aspects of the experimental setup and provide detailed information on the control of the variables. Caco-2 models and alike have indeed a number of advantages and disadvantages [Grès et al. 1998, Le Ferrec et al. 2001, Thomas et al. 2008, Adler et al. 2011]. Attention is in particular necessary in those cases for which **non-suitability of the *in vitro* model has been reported e.g. for highly lipophilic compounds, substances with poor absorption, substances with a carrier-mediated transport or when first pass metabolism is involved** [Thomas et al. 2008, Willman et al. 2004]. ECVAM sponsored a study aimed at evaluating the reproducibility (between-laboratory and within-laboratory variability) and the predictive capacity of two in vitro cellular systems — the Caco-2/ATCC parental cell line and the Caco-2/TC7 clon. The study concluded that good prediction is obtained only for highly absorbed compounds (100% correctly classified), while moderately and poorly absorbed compounds are frequently overestimated [Prieto et al. 2010].

In the case of **oral-to-inhalation extrapolation** and in the absence of route-specific bioavailability information, a **correction factor of 2** was proposed (i.e. the absorption percentage for the starting route is half that of the end route). The inclusion of this factor 2 means for example that 50% (instead of 100%) absorption is assumed for oral absorption. For inhalation, the default is 100%.
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 [EChA 2008b].

An additional remark with regard to MoS calculations is whether such calculations are scientifically relevant for cosmetic substances 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 relevant to the risk assessment procedure for 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.

When cosmetic products are not the only source of exposure to an ingredient, but major exposure is caused by other sources (e.g. consumer products, food, environment), it is recommended to base its quantitative risk assessment upon aggregate exposure.

3-7.2 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 substance.

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 substance under study, has to be taken into account, as well as its frequency of application per day. 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/cm^2, 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 (F ≥ 1)
- 60 kg = default human body weight

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:

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

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 (for calculated relative daily exposure levels for different cosmetic product types, see Table 3, section 4-2).
- C (%) = Concentration of the substance 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[2]

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 substance will have to be assumed. This conservative

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

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

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).

3-7.3 MoS for children – Need for an extra safety factor?

Already in 2002, the SCCNFP issued an opinion on the calculation of the MoS for children. The question raised at that time 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 changes from 0 to 10 years and 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 1.9, whereas a higher factor of 3.2 is generally foreseen by the WHO for the variability in human kinetics (See 3-7.1). Consequently the inter-individual variation is already taken into account by the generally accepted threshold value of 100 for intact skin. Thus there is no need for an additional uncertainty factor for children when **intact skin** is involved [SCCNFP/0557/02].

This point of view is also taken by the SCCS. Risk assessment in the specific case of "children" was further discussed on the occasion of the use of parabens as preservatives in cosmetic products [SCCS/1446/11].

Definitions

As "children" are developing organisms at various stages of immaturity and maturation up to nearly two decades with age-dependent different susceptibilities and sensitivities [Makri et al. 2004; Lemper et al. 2009] compared to adults, for clarity reasons it seems necessary to define a number of age-related terms, usually covered by the word "children":

- full-term neonate < 1 week
- newborn 1 week – 2 months
- early infant 2 – 6 months
- crawlers/toddlers 6 months – 2 years
- preadolescent 2 – 12 years
- adolescent 12 – 18 years

Age-related susceptibilities/sensitivities

The necessity of an additional safety factor for the different age groups beyond the usual factor of 100 has been extensively discussed in the scientific literature [Renwick et al. 1998 and 2000; Makri et al. 2004]. A number of potential risk factors do exist in the newborn and early infant. They are *in extenso* described in Annex 1 of SCCS/1445/11, but as dermal exposure in children is a topic of high importance for several cosmetic substances, the most important points are summarized here.

Dermal exposure of the newborn and early infant[1]

- A full-term baby possesses all skin structures of adult skin, and anatomically these structures do not undergo dramatic changes after birth. **The dermal absorption in newborn skin is similar to that observed in adult skin, when the skin is intact.**
- For babies during their first weeks and months, however, a number of typical differences and potential risk factors exist which are not present in the adult. These are:

(i) The surface area/body weight ratio (mentioned above) is 2.3-fold higher in newborns than in adults, changing to 1.8- and 1.6-fold at 6 and 12 months, respectively. This ratio is covered by the intraspecies factor of 10 used in exposure-based risk assessment (in MoS).

(ii) Toxicokinetic parameters differ between various age groups of children and adults and can result in reduced clearance and/or longer half-life that might either increase or decrease the potential risk of an adverse reaction on babies, depending on the toxicity [Renwick et al. 2000]. After the neonatal period half-life is decreasing below adult levels.

For the CYP450s in the liver, lower activities in children as compared to adults have been described [Johnson 2003]. This data suggests that the extent of bioactivation in children between one and ten years will unlikely be higher as compared to adults, indicating that a specific safety factor for age-related differences in toxicokinetics is not required [SCCS/1486/12].

With respect to skin metabolism, it is recognized that some metabolic enzymes seem to be lower expressed in the skin of children, in particular under the age of 1 year. Hence, neonates, newborns and early infants might have higher internal exposure to certain cosmetic substances than adults. For a sound risk assessment, relevant human data regarding metabolism is necessary. This data could for instance be gained by an approach combining *in vitro* data on the metabolism of the compound under investigation and toxicokinetic modeling. For toxicokinetic modeling of the biotransformation in humans of different age groups, relevant *in vitro* data regarding phase I and phase II biotransformation are needed in both human skin and liver [SCCS/1446/11].

(iii) *In–use* conditions of topical products should be considered in exposure-based risk assessment of the finished product. It should be noted that comprehensive exposure data for newborns and early infants are not available in the open literature.

(iv) The nappy area: the skin barrier function in the nappy area and non-nappy regions are indistinguishable at birth but show differential behavior over the first 14 days, with the nappy region having **a higher pH and increased hydration**. With respect to *skin hydration* in the nappy zone, newborns tend to have somewhat higher water content in the horny layer and a greater variation than newborns, infants and crawlers up to one year. The pH is kept at a slightly acidic range of 5-6, which is similar to that in the adult. However, the nappy area is susceptible to inflammation and the buffering capacity is compromised. This may occur in particular between 6-12 months of age - **so-called nappy dermatitis**, which consists of episodic acute skin inflammation (mean duration 2 to 3 days) caused by physical, chemical, enzymatic, and microbial factors in the nappy environment, for example it is seen with diet switches *(*breast feeding, bottle feeding, solid food).

(v) Susceptibility against micro-organisms: this is in particular the case in the nappy area and a consequence of a potentially changed barrier function in

[1] The considerations in this section refer to full-term neonates and not to premature babies still under medical care

case of damaged skin. Therefore baby cosmetics should be adequately preserved (as is the case for all cosmetics) and formulated with an appropriate pH (see also under 4-4.1).

With respect to points (i) - (iii) above, there is no need for a general additional uncertainty factor for children when intact skin is involved. There might be the need for an additional safety factor if substance-specific data clearly demonstrate that inter-individual variability would result in a value higher than 10.

Cosmetic products used in the nappy area

In the nappy area special circumstances are present resulting from the close confining clothes and nappies, uncontrolled urination and defecation and resulting problems with potential damage of the skin in the nappy zone. Modern nappy technology has shown to provide increasingly good skin compatibility, leading to a decline in the frequency and severity of nappy dermatitis. However, irritant nappy dermatitis cannot be completely avoided and might have an impact on dermal absorption of substances.

As cosmetic products are meant to be used on intact skin medical consultation is necessary in the case of real skin damage and pharmaceutical products (and not cosmetics) should be used.

For the development of baby cosmetics and the risk assessment of products intended to be used in the nappy area, the potential impact of irritation on dermal absorption of the chemical needs to be considered by the safety assessor in the final quantitative risk assessment of their products.

From the above, the following two main conclusions can be drawn:

- The skin structure of full-term neonates/newborns and early infants is similar to that of adult skin and the dermal absorption is comparable. However, distinction should be made between the skin of the nappy zone and the rest of the baby skin, since for this particular area risk factors exist which are not present for the rest of the body. Therefore, the nappy zone should be further considered, independent of the substance(s) under question.

- The SCCS is of the opinion that in general no additional safety factor needs to be included for substances used in children's cosmetics used on intact skin as an intra-species assessment factor of 10, covering the toxicokinetic (3.2) and toxicodynamic (3.2) differences between children and adults, is already included in the MoS calculated for individual substances.

3-7.4 Assessment of 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.

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.

Non-genotoxic carcinogens

In case 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.

Genotoxic carcinogens

Both the Scientific Committees [SCs, 2009] and REACH [ECHA, 2008] have concluded that risk assessment of compounds that are as well genotoxic as carcinogenic should be done on a case by case basis. Whenever sufficient information is available, an appropriate dose descriptor, T25 or BMDL10, should be identified. The dose descriptors represent the chronic dose rates usually expressed as mg per kg bodyweight per day. The T25 is defined as the dose which will give tumours at a specific tissue site in 25% of the animals after correction for spontaneous incidence and within the standard life time of the species [Dybing et al., 1997]. The determination of BMDL10 uses mathematical curve fitting techniques to calculate the lower 95% confidence level at a 10% benchmark dose [EFSA, 2005]. Both T25 and BMDL10 can be used as a starting point to determine an additional Lifetime Cancer Risk (LCR) or to calculate a Margin of Exposure (MoE), which represents the ratio between a dose descriptor and the estimated human exposure dose.

The Lifetime Cancer Risk approach
Three methods for calculation of LCR 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 (LED10) (equivalent to BMDL10) method" has been used more recently by the US EPA [1996b] and the "T25 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 T25 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 T25 is determined. The determination of T25 is described in detail in EC [1999] and Dybing et al. [1997].

The animal dose descriptor (T25) is converted to the human dose descriptor (HT25) based on comparative metabolic rates, by using the following formula [Sanner et al. 2001]:

$$HT25 = \frac{T25}{(\text{body weight}_{human}/\text{body weight}_{animal})^{0.25}}$$

Based on the daily lifetime systemic exposure dosage (SED), the lifetime cancer risk is calculated by linear extrapolation by use of the following formula:

$$\text{Lifetime cancer risk} = \frac{SED}{HT25 / 0.25}$$

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 organizations have considered that an LCR in the general population of less than 10^{-5} is of little or no concern [SCCS/1486/12].

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..

Dose-response relationships: if the available 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 T25s 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 or does not occur daily e.g contaminants in hair dyes the average daily dose should be corrected according to the frequency of exposure [SCCNFP/0797/04, SCHER/SCCP/SCENIHR 2009, EChA 2008b] (e.g. for a permanent hair dye used once per month, the estimated exposure dose is divided by 30).

The Margin of Exposure (MoE) approach

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) (MoE = BMDL10 (T25)/SED). Depending on the quality of the animal carcinogenicity data and the number of dose levels used in these studies, the dose-descriptors BMDL10 or the T25 are applied as dose descriptors.

EFSA [2005] concluded "that a MoE of 10,000 and above, based on a BMDL10, or 25,000 and above, based on T25 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 a factor of 100 to allow for inter- and intraspecies differences and an additional factor of 100 to allow for the uncertainties covered under inter-individual human variability in cell cycle control and DNA repair. According to quantitative risk characterisation based on the

T25 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 without long-term animal carcinogenicity studies

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 lowest effective dose (LED) giving a response in an *in vivo* genotoxic test after oral or inhalation exposure and the carcinogen dose descriptor T25, 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/T25 was equal to 1.05 and that for 90% of the substances the numerical value of LED was similar to the numerical value of T25 within a factor of less than 5–10. The results suggest that determination of LED for *in vivo* genotoxicity could probably be used in a semi-quantitative method for risk assessment of mutagens without a long-term animal carcinogenicity study. The finding has recently been confirmed in a study from the Netherlands [Hernandez *et al*., 2011].

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]. Cosmetics Europe (formerly 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 to regulate all hair dyes listed as substances 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].

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].

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 substances, 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 in March 2010 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, exposure during a day may be very variable (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 Assessment 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 substances 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 the International Agency for Research on Cancer (IARC): The Working Group considered the epidemiological evidence inadequate, and concluded that personal use of hair colourants 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.

3.9 THE THRESHOLD OF TOXICOLOGICAL CONCERN (TTC)

3-9.1 General concept of TTC in risk assessment

The use of the TTC approach for cosmetics and consumer products has been evaluated by the SCCS/SCHER/SCENHIR [SCCP/1171/08].

The TTC concept is a risk assessment tool trying to identify exposure levels below which no toxicity is expected to occur. Currently, it is 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 more than 1500 chemicals (Carcinogen Potency Database, CPDB) [Gold et al. 1984] and one database containing 613 chemicals based on other toxicological endpoints (Munro database) [Munro et al, 1996] are available. Both are based on systemic effects after oral exposure.

Application of the TTC approach in risk assessment in any area requires a high level of confidence in: 1) the quality and completeness of the databases; 2) the reliability of the exposure data for the intended uses of the compound under study; and 3) the appropriateness of any extrapolations. It is the opinion of the Scientific Committees that in each of these areas further research is needed.

3-9.2 TTC approach for human health risk assessment of chemical substances

The Scientific Committees (SCs) consider the TTC approach, in general, scientifically acceptable for human health risk assessment of systemic toxic effects caused by chemicals present at very low levels. The application of the TTC should be done on a case-by-case basis and requires expert judgement. The TTC approach is not applicable for a number of chemical classes, which are indicated in detail in SCCP/1171/08 (adopted in 2012).

Practical application of the TTC approach to chemicals with no genotoxicity alert is usually done by analysing the chemical structure and using Cramer classification as indicator of systemic toxicity. Recent analyses have revealed a number of misclassification of compounds when using the Cramer decision tree in its present form.

The SCs conclude that the TTC value of Cramer Class II is not supported by the presently available databases and these substances should be treated as Class III substances. The SCs accept in principle the division into Cramer Classes I and III. When assigning a chemical to the lowest toxicity class **(Class I, 1800 µg/person/d corresponding to 30 µg/kg bw/d for substances with no genotoxicity alert), classification should be carefully considered and justified. If classification in Class I cannot be justified, the SCs recommend a general default value equivalent to Cramer Class III compounds (90 µg/person/d corresponding to 1.5 µg/kg bw/d for substances without genotoxicity alerts)**. All the scientific information available today should be used to define the various toxicity classes before expanding their number, i.e. the classification scheme should be modified based on up-to-date toxicological knowledge.

For the moment, **the default value of 0.15 µg/person/d corresponding to 2.5 ng/kg bw/d** can be used **for chemicals with genotoxicity alerts** and hence possible DNA reactive carcinogens, but its scientific basis should be strengthened. This could be achieved by e.g. extending the database, analyzing all available carcinogenicity studies, using allometric adjustment factors and/or using the T25 or 1, 5 or 10% benchmark dose as points of departure for linear extrapolation.

Usually, TTC values are expressed as an amount per person per day. In order to be applicable to the entire population, including all age groups, it is advised to express TTC values in an amount per body weight per day and give special consideration to infants under the age of 6 months because of the potentially immature metabolism for some chemicals structures, in particular when the estimated exposure is close to tolerable exposures defined by the TTC values.

3-9.3 TTC approach for human health risk assessment of cosmetic products, consumer products and others

In a regulatory context, the TTC concept is presently applied only in very low exposure situations. From a scientific perspective the TTC approach can be applied to cosmetics, other consumer products and chemicals to which consumers may be exposed. However, the TTC approach relates only to systemic effects and, at present, **cannot be used for the assessment of local effects. Allergy, hypersensitivity and intolerance are excluded due to uncertain dose-response relationship.**

In relation **to cosmetic substances, the databases currently in use require further development and validation.** From a scientific point of view, there is no distinction between intentionally added substances or inadvertent contaminants. The applicability of the TTC concept for both types of substances is primarily dependent on exposure conditions, chemical structure and the databases available. **For cosmetic substances, the TTC concept can only be used for those compounds which belong to a sufficiently represented structural class in the TTC database and where appropriate exposure data are available.**

In addition, it should be noted that an appropriate exposure assessment is essential for all risk assessments, including application of TTC. Significant exposure is likely for consumer products, especially when they are frequently used. This may involve oral exposure (e.g. mouthing), skin contact and/or exposure via inhalation by using e.g. toys, cosmetics or cleaning products.

3-10 ASPECTS TO CONSIDER WITH RESPECT TO THE RISK ASSESSMENT FOR THE INHALATION ROUTE

In general, exposure to cosmetics and their ingredients occurs via the skin. Products like body lotion, soaps etc. are topically applied and penetration via the skin is the main route of entry to the body. However, for a number of cosmetic products, inhalation is (also) a potential entry. This is the case for instance when substances with a high vapour pressure are used, like solvents in e.g. nail polish remover. Another example is the increasing use of cosmetic products in spray form, like deodorants, hair spray, but also sunscreens. For these products, the primarily dermal exposure has to be evaluated [Steiling et al. 2012], but in case exposure via inhalation is significant, this has to be also taken into account in the risk assessment for both possible local effects as well as potential impact on the body burden.

For the total systemic exposure, all relevant exposure routes (including the possible uptake via the skin, the respiratory tract or even via the oral uptake) need to be considered for the final risk assessment.

For **an appropriate assessment of the toxicity via inhalation**, knowledge of the **hazard profile of the ingredients**, **their concentrations in the final product** as well as the **exposure pattern of the final product** is needed. An overview of the evaluation of the safety of cosmetic substances in spray products is given by Rothe et al. [2011]. In Fig. 4, the basic principles for the safety evaluation of inhalable cosmetic products and their ingredients are provided.

3-10.1 Hazard assessment for the inhalation route

For the inhalation route no validated *in vitro* methods exist. A number of human-based reconstructed tissue models for the respiratory tract are commercially available but until now their use in hazard/risk assessment is very limited, one of the reasons being the different regions of the airway tract with different functionality [Sauer et al. 2013]. Therefore, for hazard assessment, data on local and systemic toxicity has to be considered.

a) Local respiratory tract toxicity

The toxic effects of a chemical on the respiratory tract can be assessed based on an inhalation study. Suitable surrogates for data on local effects to the respiratory tract

could be information on irritancy to eyes and mucous membranes (*in vivo* and *in vitro*).

b) Systemic toxicity
In a standard toxicological dossier for a cosmetic substance, there are not much data available with regard to the inhalation route, although an acute (LC_{50}) study might be available (REACH), or an inhalation study with repeated exposure. From these studies, information on the intrinsic properties of the substance via the inhalation route can be obtained.

3-10.2 Exposure assessment for the inhalation route

A spray product may be released as a vapour or as an aerosol. For exposure to aerosols, the size of the particles/droplets to which the consumer is exposed determines the extent of exposure. Particles/droplets with a size >10 µm are trapped/impacted/filtered in the nose, mouth, throat or tracheobronchial area. Only particles/droplets with a size <10 µm are small enough to reach the deeper part of the lungs, where they can enter the alveoli and may become systemically available.

Generally, there are two types of spray applications: propellant driven aerosol sprays and pump sprays. According to Bremmer et al. [2006a, 2006b], **propellant driven aerosol sprays are often developed to produce a fine mist, with often a relevant fraction of particle size <10 µm**, compared to pump sprays, which in general produce larger particles/droplets.

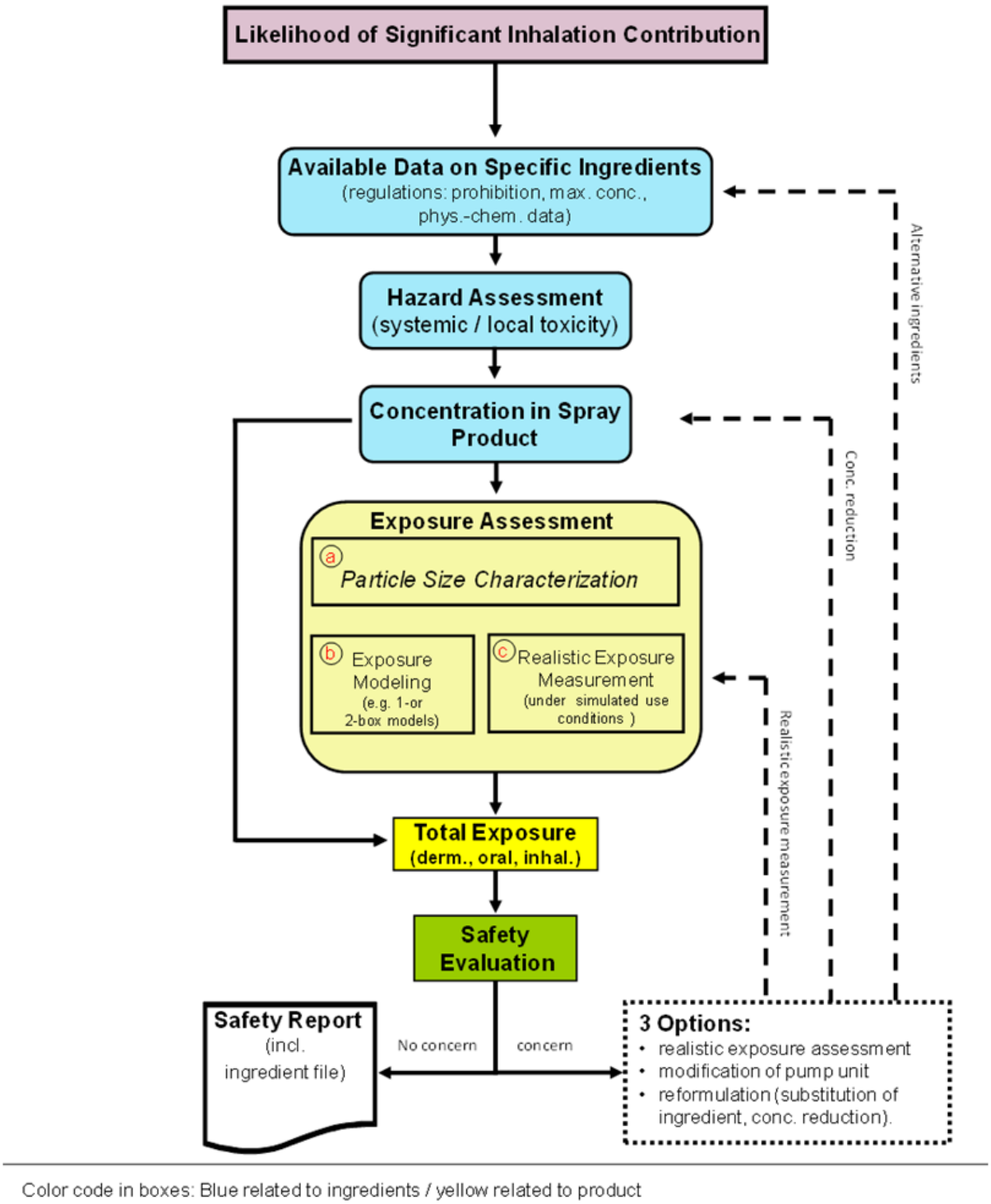

Fig. 4: Basic principles for the safety assessment of inhalable cosmetic products and their substances.

The size of the droplets is influenced by the actual formulation (surface tension) including the individual use of solvents and propellants. They are as well related to the geometry of the can spray nozzle and can size. **Information on the realistic particle/droplets size distribution is important in the safety assessment of a cosmetic spray product** as it determines the depth of penetration of the substance into the respiratory tract.

The level of exposure can be directly measured under standard exposure conditions, or by using mathematical models. When measuring exposure, it is important to measure during the relevant exposure period after spraying, under relevant conditions [Carthew

et al. 2002]. The REACH guidance [EChA, 2010] provides equations that can be used as a conservative approach. For a more realistic assessment, higher tier models like the ConsExpo model can be considered [RIVM 2012] as a first estimate.

3-10.3 Safety assessment of substances in cosmetic products in spray form

For safety assessment, the modelled or measured consumer exposure to the sprayed substance/product is compared with the dose considered to be without any toxicological adverse effect based on the outcome of standard toxicological tests.
In this context, one key parameter is the No Observable Adverse Effect Concentration (NOAEC). In case such NOAEC is not available, a route to route extrapolation from oral studies with repeated applications may be applicable [EChA, 2010]. Information obtained for the oral route may be considered to be extrapolated to the inhalation route on a case by case basis for systemic effects.
Depending on the outcome of the safety evaluation, there may be a need to refine exposure assessment (e.g. if based on a conservative approach), to modify the spray characteristics by using different technical equipment (e.g. spray nozzle) or to reformulate the product.

It should be noted that the safety of the final cosmetic product belongs to the responsibility of the manufacturer, first importer or marketer [Dir. 76/768/EEC] **or the responsible person in the Recast** [Reg. 2009/1223/EC].

3-11 HUMAN BIOMONITORING

3-11.1 Definition

Human biomonitoring (HBM) is a systematic continuous or repetitive activity for the collection of biological samples for analysis of chemical substances, metabolites or specific non adverse biological effects to assess exposure and health risk to exposed subjects, comparing the data observed with reference levels and - if necessary - leading to corrective actions [Zielhuis, 1984].

3-11.2 Fields of application

Initially, HBM was applied at the workplace in order to complement external exposure measurements with internal exposure data, as a proof of systemic bioavailability and as a basis for decision-making with respect to the necessity of measures to reduce or minimize exposure. Subsequently, population-based HBM has emerged with the primary aims to (i) investigate the possible association between internal exposure to certain substances (e.g. due to environmental exposure) and human health status and (ii) investigate trends of exposure in the human population.

Progress – especially in the analytical field – lead to the development of sensitive, specific, reliable and robust analytical methods to determine chemical substances or their metabolites in a variety of human biological matrices down to the pg/l level [Angerer et al. 2007; Needham et al. 2007]. The concentrations measured in human body fluids can be used as indicators for the dose taken up under real-life exposure conditions in the relevant species and population e.g. to assess human exposure (see SCCS/1446/11 on parabens). It should, however, be kept in mind, that **HBM accounts for all sources (air, water, diet, consumer products etc.) and all routes of uptake**.

Thus, HBM data as such are not suitable for the assessment of exposure of a (cosmetic) substance when other (non-cosmetic) sources for uptake and exposure are involved. They rather should be used **as support in risk assessment and risk management**. However, back-calculation from biomonitoring data to external exposure data requires additional information (e.g. type of biomarker, exposure

scenarios, toxicokinetic behavior, biochemical mechanisms and toxicokinetic modelling), described in depth in a recent publication [Tan et al, 2012].

As an approach to assess exposure and health risk limit values , Human Biomonitoring Values (HBM-Values) [Kommission HBM 1996], Biological Exposure Indices (BEI) (ACGIH[1]) or Biological Equivalents (BE) [Hays et al, 2008] are evaluated by various committees. These are reference values, which are a statistical description of the inevitable background exposure of the general population (95th percentile) to a certain substance. In this respect, HBM results may provide information whether exposure to consumer products and their components give rise to health concern or not.

If adequately applied (i.e. toxicokinetics and metabolism of a substance is taken into account), HBM data can support and complement information on all aspects of ADME of a cosmetic substance, which are addressed in the safety evaluation dossier (e.g. results from *in vitro* and *in vivo* dermal absorption studies, results from toxicokinetic studies); HBM may also complement the results of further *in vitro* methods and animal studies, which are usually used for exposure assessment and for risk assessment in registration procedures. Especially in view of the prohibition of *in vivo* animal studies on cosmetic substances, HBM enables to gain important *in vivo* information, also directly in humans (no inter-species extrapolation, limited number of people involved). Ethical restraints usually do not pose a problem. If sufficient animal data are available, intraspecies variation can also be addressed using HBM.

3-11.3 Limitations

When using HBM in the context of safety evaluation of consumer product ingredients, aspects which limit its field of application should be taken into account:

- HBM is applicable to substances that are systemically taken up and where the half-life of the biomarker enables sampling and analytical determination.

- HBM is not appropriate when the relevant biomarker is an endogenously formed substance, present in much higher concentrations than those caused by uptake from the environment or consumer products.

- Various factors influence HBM results , including age, gender, lifestyle, consumer habits, diet, place of residence etc. as they modify the amounts of chemical substances taken up. Inter-individual differences in the metabolism of chemical substances, excretion of metabolites, health status as well as different compositions of biological materials like varying dilutions of urine etc., even under identical conditions of exposure, may provide different HBM results .

- Other error sources are contamination of samples during collection and handling of the biological samples [Calafat and Needham, 2009].

3-11.4 Conclusion

HBM can estimate the amounts of chemical substances that have been taken up in the human body. It therefore enables the measurement of internal exposure to absorbed chemical substances or their metabolites. HBM does not replace other exposure methods such as the determination of chemical substances in environmental media, consumer product ingredients etc. nor does it replace toxicological testing and SED calculation, but **it complements** these methods. HBM moreover can **give some insight in human ADME of chemical substances, being important for safety evaluation in particular as animal experiments are banned in a number of cases**. Ethical aspects of HBM have to be handled according to national/international rules.

[1] American Conference of Governmental Industrial Hygienists

HBM may be a useful tool for the safety evaluation of consumer product (cosmetic) ingredients. The extent to which it can be used is governed by the question to be addressed for the specific substance under consideration and by the limitations of the technique.

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 requirements of the recast [2009/1223/EC], a product information file (PIF) must be kept available by the responsible person 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 a safety assessment (Cosmetic Product Safety Report), made by a safety assessor, with the competence as required in Regulation (EC) No 1223/2009 (Art. 10.2) and being responsible for it. The safety evaluation of the finished product is based upon the toxicological profile of the substances, their chemical structure and their exposure level. In the "Guidelines on Annex I to Regulation (EC) No 1223/2009 on the Cosmetic Product Safety Report" it is explained in detail how a CPSR should be established.

It must be emphasised that it remains the responsibility of the safety assessor to justify whether enough information on the substances, 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 substances, 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 3-5.6),
- the substances 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 (see also section 3-8).

Every specific exposure scenario will be linked to a certain amount of substance that may be ingested, inhaled or absorbed through the skin or mucous membranes. Translated into a daily amount per kg body weight, it is considered the 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 substance may be used,
- method of application: rubbed-on, sprayed, applied and washed off, etc.,

- concentration of the substance 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 sensitization 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 SED, an important parameter for calculating the MoS of substances 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/cm^2, 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 (F ≥ 1)
- 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 substance 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 et al. 2005] and indicate exposed skin surface areas per cosmetic

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

product type[1]. 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[2]
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[3]		*2/day*

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

[1] 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].

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

[3] Ferrario et al. 2000.

Product type	Skin surface area involved (RIVM)		Frequency of application*
	Surface area (cm²)	Parameters (if specified)	
Deodorant			
Deodorant aerosol spray[1] and non-spray[2]	200	both axillae	*2/day*
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 (now Cosmetics Europe) studies (see next paragraphs for details on these 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 substance 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 (now Cosmetics Europe). Upon repeated request 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 Steiling et al. 2012
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.

The new figures for the daily amounts of cosmetic products are here taken up. In the Colipa (now Cosmetics Europe) 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 (now Cosmetics Europe) 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 (now Cosmetics Europe) 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 7th Revision of the 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 (Cosmetics Europe) data [SCCNFP/0321/00; 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 (now Cosmetics Europe) 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]. Under laboratory controlled conditions or under realistic conditions of tanning on the beach using own sun products (lotions, alcoholic solutions, gels, creams) applied on the whole body surface, values for use of products between 0.5 - 1.3 mg/cm² are found [Stenberg et al. 1985, Bech-Thomsen et al. 1993, Diffey B.L. 1996, Gottlieb et al. 1997, Autier et al. 2001 and 2007]. The values are depending on the study protocol used, the location on the body measured and several other factors. It is mentioned [Gottlieb et al. 1990] that in routine use even lower amounts than those documented in a supervised study may be delivered to the skin. The latter occurs for example when sunscreen is applied by the exposed person himself hurriedly or when applied to both hairy skin and areas which are difficult to reach such as the back and the lower legs. The value used in the Notes of Guidance of 18.0 g per day is derived from the application of 0.5 mg/cm² for the **entire skin surface of an adult** (17500 cm²), twice a day, resulting in 17.5 gram in total per day per person.

For some cosmetic substances, 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 and are not used in addition to all these cosmetic products at the same time. UV-A filters are often present in face creams/body lotions and these are included in the table.

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 substances 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 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]. More information on risk assessment for the inhalation route is present under 3-10.

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

4-3.1 Introduction

Until 11 July 2013, the safety assessment of a cosmetic product may be performed according to Directive 76/768/EEC and its 6th and 7th Amendments. After this date the requirements of Regulation (EC) No 1223/2009 should be fully met. In the meantime a combination of both legislations is allowed. The content of the PIF and CPSR of a cosmetic product are clarified in Art. 11 and Annex I of Regulation (EC) No 1223/2009 respectively, as well as in the Guidelines to Annex I.

Each cosmetic product is considered as an individual combination of cosmetic substances. It is generally accepted that the safety evaluation can be done by ascertaining the toxicity of its substances [93/35/EEC, 2003/15/EC, 2009/1223/EC] on the condition that the information on the most relevant toxicological endpoints of its constituent substances is available. In some cases, however, additional information on the finished product is needed in the interest of a sound safety assessment. Examples are cosmetics for specific target consumers groups (babies, sensitive skin, etc.), the presence of certain substances that increase skin penetration and/or skin irritancy (penetration enhancers, organic solvents, acidic components, etc.), the presence of a chemical reaction between individual substances 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 substance 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 substances

During the safety evaluation of a finished cosmetic product, the available toxicological data for all substances 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 substances 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 substance 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 substances, 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. Also potential leaching of substances of the packaging into the product should be investigated.

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 cosmetic product safety assessment of the finished product. This means that all toxicological data available on the individual substances 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 substance, 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 substances 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 person.

Finally, the safety of the product should be reviewed on a regular basis. To that end, undesirable and serious 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. Regulation (EC) No 1223/2009 defines undesirable and serious undesirable effects as follows:

*- **An undesirable effect** is an adverse reaction for human health attributable to the normal or reasonably foreseeable use of a cosmetic product.*

*- **A serious undesirable effect** is an undesirable effect which results in temporary or permanent functional incapacity, disability, hospitalisation, congenital anomalies or an immediate vital risk or death.*

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

The proof of qualification of the safety assessor must be included in the dossier. The safety assessor may be employed by the responsible person 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.

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. In some justified cases (e.g. alcohol content > 20%), end product testing is not necessary [ISO 29621, 2010]. 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[1] 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 of the product (cfu = colony forming unit).

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 of the product.

[1] Colipa is now called "Cosmetics Europe"

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 1 g or 1 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 1 g or 1 ml of the product. 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. 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 responsible person 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 responsible person to decide on the details of the test to be used.

4-4.4 Good Manufacturing Practice

In order to comply (mandatory but no certification required) 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).

According to Article 8 of Regulation (EC) No 1223/2009, *good manufacturing shall be presumed where the manufacture is in accordance with the relevant harmonised standards, the references of which have been published in the Official Journal of the European Union.*

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.
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Official Journal L 15, 17/01/1987 p.29.

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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.
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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.
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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.
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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.

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Official Journal L66, 11/03/2003 p.26.

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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.

2006/78/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.

2006/1907/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.
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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
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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*.

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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*.

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APPENDIX 1: LISTS OF SUBSTANCES

1. INTRODUCTION

Regulated cosmetic substances can be found as Annexes II, III, IV, V and VI to Regulation (EC) No 1223/2009. These annexes lay down clear limitations and requirements for the cosmetic substances concerned.

Another important list of cosmetic substances is the **INCI** (International Nomenclature Cosmetic Ingredient) inventory [96/335/EC] or CIN [2009/1223/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 substances called **CosIng**, (Cosmetic ingredients) which combines INCI names and synonyms of the listed substances 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] and the Recast [2009/1223/EC] directly refer 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 substances used in cosmetic products.

2. ANNEXES II, III, IV, V AND VI TO THE COSMETIC PRODUCTS REGULATION

The Cosmetic Products Regulation defines Annexes II, III, IV V and VI, which have been described in section 2-4.2.

3. INVENTORY OF SUBSTANCES USED IN COSMETIC PRODUCTS

Article 33 of Regulation (EC) No 1223/2009 states that the Commission shall compile and update a glossary of common ingredient names employed in cosmetic products [2003/1223/2009].

On 8 May 1996, the European Commission established an Inventory and a common nomenclature of the substances 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 substance under consideration.
An entry in the Inventory provides identification of that particular substance 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; now called Cosmetics Europe), 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 substance.
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 substances.

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 substances.

4. COSING - EC INFORMATION ON COSMETIC SUBSTANCES

The CosIng 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. 15** of the Cosmetic Products , 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 September 2012

6. REFERENCES

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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.
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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

Scientific Committee on Consumer Safety

SCCS

OPINION ON

.....

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 Agency (EMA), 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, Elsa Nielsen, Thomas Platzek, Suresh Chandra Rastogi, Vera Rogiers, Christophe Rousselle, Tore Sanner, Jan van Benthem, Jacqueline van Engelen, Maria Pilar Vinardell, Rosemary Waring, Ian R. White

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The opinions of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The opinions are published by the European Commission in their original language only.

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.

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External experts (if applicable):

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Keywords: ……; ……; ……; ……; ……

Opinion to be cited as: ……
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- …

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