THE SCCS NOTES OF GUIDANCE

FOR THE TESTING OF COSMETIC INGREDIENTS

AND THEIR SAFETY EVALUATION

9th revision

The SCCS adopted this guidance document at its 11th plenary meeting
of 29 September 2015

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. The emphasis of this guidance is on cosmetic ingredients, although some guidance is also given for the safety assessment of finished products. It is designed to provide guidance to public authorities and to the cosmetic industry in order to improve harmonised compliance with the current cosmetic EU legislation. An important development was the 2009 legislative recast, which transformed the cosmetic Directive 76/768/EEC into a Regulation. It is emphasised that from 11 July 2013 onwards this Regulation (2009/1223/EC) was fully applicable. The European cosmetic legislation prohibits the marketing of finished products containing ingredients or combinations of ingredients that have been subject to animal testing after 2013. Therefore the SCCS has closely followed the progress made with regard to the development and validation of alternative methods.

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 2012 (SCCS/1501/12). Since then, several new addenda, opinions and memoranda 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, scientific committees (SCHER, SCENIHR) and Cosmetics Europe (formerly Colipa) is gratefully acknowledged.

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TABLE OF CONTENTS

1. INTRODUCTION

Directive 76/768/EEC, for years the legislative framework of cosmetics and their ingredients in the EU, was replaced by Regulation number 1223/2009 in July 2013 in order to uniform the safety of cosmetics, better harmonise compliance within the Member States, simplify procedures and streamline terminology. The most significant changes introduced by the new Cosmetic Regulation include:

(1) **Strengthened safety requirements for cosmetic products** Manufacturers need to follow specific requirements in the preparation of a product safety report prior to placing a product on the market.

(2) **Introduction of the notion of a "responsible person"** Only cosmetic products for which a legal or natural person is designated within the EU as a "responsible person" can be placed on the market. The new Cosmetics Regulation allows the precise identification of the responsible person and clearly outlines his/her obligations.

(3) **Centralised notification of all cosmetic products placed on the EU market** Manufacturers will need to send product notification only once – via the EU Cosmetic Product Notification Portal (CPNP).

(4) **Introduction of reporting serious undesirable effects (SUE)** A responsible person will have the obligation to notify serious undesirable effects to national authorities. The authorities will also collect information coming from users, health professionals and others. They will be obliged to share the information with other EU countries. More information on reporting of SUE.

(5) **New rules for the use of nanomaterials in cosmetic products** More information on nanomaterials

(6) **A set of requirements for CMR** (carcinogenic, mutagenic, reproductive toxic) **substances**

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 long history and have been made for thousands of years from 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 (1223/2009/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, the Scientific Committee on Consumer Safety (SCCS). These substances are addressed in the Annexes of Regulation (EC) No 1223/2009, replacing Directive 76/768/EEC from 11 July 2013 onwards.

For the safety evaluation of cosmetic substances, all available scientific data are considered, including the physical and chemical properties of the compounds under investigation, *in silico* data such as 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*). In addition, 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.
In the present update, the state-of-the-art with respect to the validated methods of the 3R (Refinement, Reduction and Replacement) strategy of Russell *et al.* (1959), is incorporated.
In particular, the SCCS gives special attention to those alternative methods that are suitable for the safety testing of cosmetic substances. These are taken up in the appropriate sections.

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 the 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 for all substances intended to be incorporated in a cosmetic product. Even though the "Notes of Guidance" have not been written particularly for the latter purpose, they can indeed be of practical use in making a PIF (product information file) for a finished cosmetic product as currently required by Regulation (EC) No 1223/2009, Annex I.
The "Notes of Guidance" should not be seen 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.

The safety evaluation of cosmetic substances and finished products remains a scientific exercise that can only be performed on **a case-by-case basis**.

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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.*

When major deviations from standardised protocols/procedures in the safety evaluation process occur, a scientific justification is essential.

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

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, manufacturing, 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 the 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.
(http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04/index_en.htm, consulted September 2015)

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.

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, consulted September 2015)

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

[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*.

Pool were published in the *Official Journal* of the European Union[1]. In 2013, the three above-mentioned Scientific Committees were renewed[2].

Finally, a new Commission Decision (C(2015)5383)[3] was adopted on 7 August 2015, establishing two scientific committees:
(a) the Scientific Committee on Consumer Safety (**SCCS**); and
(b) the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER).

2-2 MANDATE

The mission of the Scientific Committees is defined in Commission Decision (C(2015)5383), which states that they shall 'provide the Commission with scientific advice and risk assessment in the areas of public health, consumer safety, environmental risks, including, when relevant, identification of research needs to address critical information gaps, assessment of proposed future research actions and of research results'.

The SCCS on request of Commission services shall provide opinions on questions concerning health and safety risks, notably chemical, biological, mechanical and other physical risks, of:
(a) non-food consumer products such as
- cosmetic products and their ingredients, including nanomaterial, hair dyes, fragrance ingredients;
- personal care and household products such as detergents; toys, textiles, clothing, etc.
(b) services such as 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;
- rapid advice on the state of scientific knowledge concerning specific risks in case of urgent risks;
- the identification of research needs to address critical information gaps, to assess proposed future research and to assess research results in relation to the subject areas covered by its fields of competence;
- to be part of thematic networks or events with other Union bodies or scientific organisations, in order to monitor and contribute to the development of scientific knowledge in the fields of competence.

Also, upon its own initiative, the Committees shall draw the Commission's attention to a specific or emerging problem falling within its remit, which is considered to pose an actual or potential risk to consumer safety, public health or the environment.
Finally, in agreement with the Commission, the Committees shall adopt their methodology for performing and providing risk assessment and keep it under review to reflect all relevant scientific factors. They shall ensure that the methodology reflect current risk assessment practice.

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[4], whereas it is not compulsory in the domain of other non-food products.

[1] **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] **Commission Decision 2013/1297** of 11 March 2013 on the appointment of the members of the Scientific Committees set up by Commission Decision 2008/721/EC. http://ec.europa.eu/health/scientific_committees/docs/com_2013_1297_en.pdf
[3] http://ec.europa.eu/health/scientific_committees/docs/call_2015_5383_decision_with_annexes_en.pdf
[4] See Article 31 of Regulation (EC) No 1223/2009

In the preamble of Regulation (EC) No° 1223/2009 different tasks for the SCCS are mentioned in several recitals:

(28) safety assessment of hair colorants (in annex IV),
(30) providing guidance in cooperation with relevant bodies on test methodologies which take into account specific characteristics of nanomaterials,
(32) continuously reviewing the safety of CMR substances, so that substances clarified as CMR 2 or CMR 1A or 1B can be used in cosmetics under well-restricted conditions when such use for CMR 1A and 1B has been found safe by the SCCS,
(34) taking into account the exposure of vulnerable population groups,
(35) giving opinions on the safety of use of nanomaterials in cosmetic products,
(42) consultation by the Commission as regards the applicability of validated alternative methods to the field of cosmetic products,
(49) identification of substances likely to cause allergic reactions in order that their use can be restricted and/or certain conditions can be imposed,
(61) providing assistance to the Commission as an independent risk assessment body.

The compulsory consultation of the SCCS is taken up under:
Art. 15, 2(d) and 3 for substances classified as CMR substances
Art. 16, 4 and 5 for nanomaterials
Art. 18, 2 for animal testing methodology
Art. 20, 2 for setting criteria for product claims
Art. 27, 3 for determination whether the provisional measures taken with respect to the safe clause are justified or not
Art. 31, 1 for amending Annexes II to VI for safety concerns
Art. 31, 2 for amending Annexes II to VI, VIII for technical and scientific progress
Art. 31, 3 for amending Annex I to ensure the safety of cosmetic products placed on the market.

Newly introduced modifications and improvements in the current structure and working procedures of the SCCS and the other Scientific Committee can be found in Commission Decision of 7 August 2015 (C(2015)5383).

2-3 RULES OF PROCEDURE

The Rules of Procedure of the SCCS, SCHER and SCENIHR were jointly adopted by the Scientific Committees on 11 April 2013[1].
The relevant Rules of Procedure will be amended according to the Commission Decision C(2015)5383 establishing two Scientific Committees in the field of public health, consumer safety and the environment for the period 2016-2021 (SCCS and SCHEER).

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 external experts (either from the Database of Experts[2] or via a specific call[3]). Working groups, for example, deal with: Cosmetic Substances (individual substance evaluations, with the exception of hair dyes & fragrances), Hair Dyes & Fragrances, Methodologies (alternative methods and Notes of Guidance), Nanomaterials and other topics according to the needs.

The mandate on a specific substance or other issue is officially adopted by the members during a plenary meeting and published[4].

[1] http://ec.europa.eu/health/scientific_committees/docs/rules_procedure_2013_en.pdf
[2] http://ec.europa.eu/health/scientific_committees/experts/database/index_en.htm
[3] http://ec.europa.eu/health/scientific_committees/open_consultation/index_en.htm
[4] http://ec.europa.eu/health/scientific_committees/consumer_safety/requests/index_en.htm

A Rapporteur is nominated (SCCS member or external expert). Once the participants of the Working Groups have agreed on a final version of their opinion/scientific report(s), they present it to the next SCCS plenary meeting where members adopt the texts. In particular cases, an opinion may also be adopted by written procedure. The adopted opinions, once edited, are published on the Commission's website[1] for a commenting period of a minimum of 4 weeks to allow the applicant, and other stakeholders as well, to send their comments that are subsequently considered by the SCCS [2] and, when considered appropriate, incorporated in a revised version[3] of the opinion. The revised opinion is published on the website, with the date of the adoption of the revised text on the right top corner, and replaces the previous version. The final opinions are not subject to further comments or revision requests. Any new data should be submitted directly to the responsible Commission unit mandating the SCCS (see box in Section 3-2).

This method of working with subgroups not only lightens the workload of the members of the SCCS, but equally and importantly, facilitates discussion of the individual topics with the appropriate experts in the field of interest, thus enhancing 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 were published on the Internet and can be accessed through the Committee's website[4]. All SCCP / SCCS opinions can easily be located through the ingredient's substance category 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.

This is done through the Notes of Guidance for testing of cosmetic ingredients and their safety evaluation that are regularly revised and updated in order to incorporate new knowledge and scientific advances. Therefore, submitted dossiers should be in accordance with the latest published version. The 8th Revision SCCS/1501/12 is now replaced by the 9th Revision SCCS/1564/15.

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 testing procedures to be followed for chemical substances include not only *in vivo* animal models but also *in vitro* models. Furthermore, testing procedures in accordance with the OECD (Organisation for Economic Co-operation and Development) Guidelines, and, on a case-by-case basis, well documented scientifically

[1] http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
[2] http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm#page1
[3] http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm#page2
[4] http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04/index_en.htm

justified methods based on *in vitro* models or other 3R-alternative procedures, are also carefully considered.

Over the years, several 3R-alternative methods have been developed and validated. These are commonly taken up in Commission Regulation (EC) No 440/2008. The latter includes Reduction and Refinement and Replacement methods. Given the fact that Regulation (EC) No 1223/2009 imposes the use of validated replacement methods not only for finished cosmetic products but also for their ingredients, much attention is given to the use of validated 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 240 opinions to that list. The majority of these opinions were implemented in the 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.

REGULATION (EC) NO 1223/2009	
Annex I	Cosmetic Product Safety Report
Annex II	List of Prohibited substances
Annex III	List of Restricted substances
Annex IV	List of colourants
Annex V	List of Preservatives
Annex VI	List of UV-filters
Annex VII	Symbols used on packaging/container
Annex VIII	List of validated alternative methods to animal testing
Annex IX	**Part A** Repealed Directive with its successive amendments **Part B** List of time-limits for transposition into national law and application
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 eyelashes*****.** 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, 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 testing skin sensitising potential	*Examples*
- classification of skin sensitisers and grading of test reactions	SCCP/0919/05
Alternative methods in the safety assessment of cosmetics	
- 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	
- 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	
- new CMR classification according to Regulation 790/2009	SCCP/0913/05
- CMR Guidance (see Section 3-7 and Appendix 5)	SCCS/1284/09
Safety assessment of hair dyes and colourants	
- hair dyes and skin sensitisation	SCCP/1104/07 SCCS/1509/13
- hair dye substances and hydrogen peroxide used in products to colour eyelashes	SCCS/1475/12 SCCS/1553/15
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	
- parabens	SCCS/1446/11 SCCS/1514/13
- products resembling food and/or having child-appealing properties	SCCS/1359/10
- nitrosamines in balloons	SCCS/1486/12
Fragrance allergy in consumers	
- sensitisation quantitative risk assessment (QRA)	SCCP/1153/08
- fragrance allergens in cosmetic products	SCCS/1459/11
Nanomaterials	
- nanomaterials in cosmetic products	SCCP/1147/07
- safety assessment of nanomaterials in cosmetics	SCCS/1484/12
- relevance, adequacy and quality of data on nanomaterials	SCCS/1524/13
- term "sprayable applications/products" for nanomaterials	SCCS/1539/14

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
- genotoxic and carcinogenic substances	SCHER/SCCP/ SCENIHR (2009)
- Threshold of Toxicological Concern (TTC)	SCCP/1171/08
- potential endocrine disrupting/modifying substances	SCCS/1544/14

3. SAFETY EVALUATION OF COSMETIC INGREDIENTS

3-1 INTRODUCTION

The safety of cosmetic products is based on the safety of the ingredients
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).

The safety of cosmetic ingredients is evaluated by toxicological testing. Until recently, this was mainly done by using experimental animals. Deadlines for animal testing, however, are imposed and laid down in Directive 2003/15/EC, the 7th Amendment of Cosmetic Directive 76/768/EEC, making the use of validated alternative replacement methods in toxicological validated testing compulsory. These deadlines are meanwhile in force in the Cosmetics Regulation (EC) No 1223/2009 and therefore in principle only replacement methods are allowed in the EU. Guidance on how to comply with the animal testing ban and marketing ban can be found in the 50th recital of the Regulation, in Commission Communication (COM/2013/135), a factsheet of ECHA and the 2nd ECHA report on the use of alternatives to testing on animals.

The 50th recital of Regulation 1223/2009 states the following: *"it should be possible to take into account results of risk assessments that have been carried out in other relevant areas. The use of such data should be duly substantiated and justified."*
Commission Communication COM/2013/135 further elucidates: *"If animal testing was involved and took place after the 2013 deadline, the product information file should allow verification on whether the testing was carried out in order to meet the requirements of the Regulation or for other purposes. To this end the file should contain documentation on any use of the substance in products other than cosmetic products (product examples, market data etc.), as well as documentation on compliance with other regulatory frameworks (e.g. REACH or other legal frameworks) and a justification of the need for the animal testing under that other framework (e.g. testing proposal under REACH)"*.

A factsheet[1] has recently been published clarifying the practical meaning and implications of the Commission Communication in the context of REACH. The interface between REACH and the Cosmetics Regulation has been illustrated in a scheme, see **Appendix 3**. It has to be noted that the Cosmetics Regulation does not restrict testing under REACH, if: a) this testing is required for environmental endpoints; or b) the substance is also registered for non-cosmetic uses. Even if a substance is registered exclusively for cosmetic use, the animal testing requirements continue to apply to tests needed to assess the risks from exposure to workers in the Chemical Safety Assessment (ECHA, 2014a)[2].

Additional recent information regarding the REACH legislation in the context of alternative methods can be found in the second report "The Use of Alternatives to Testing on Animals for the REACH Regulation", under Article 117(3), available online (http://echa.europa.eu/documents/10162/13639/alternatives_test_animals_2014_en.pdf).

ECHA has excluded from the scope of this report substances that are used in cosmetic products and fall under the scope of the Cosmetics Regulation (EC) No 1223/2009.
However, an option for derogation from the animal testing ban is foreseen in the Cosmetics Regulation, Art. 18, No 2, paragraph 6 in combination with Art. 18, No. 1 (d):

1. Without prejudice to the general obligations deriving from Article 3, the following shall be prohibited: …..
(d) the performance within the Community of animal testing of ingredients or combinations of ingredients in order to meet the requirements of this Regulation.
2. (Paragraph 6):

In exceptional circumstances, where serious concerns arise as regards the safety of an existing cosmetic ingredient, a Member State may request the Commission to grant a derogation from paragraph 1. The request shall contain an evaluation of the situation and indicate the measures necessary. On this basis, the Commission may, after consulting the SCCS and by means of a reasoned decision, authorise the derogation. That authorisation shall lay down the conditions associated with this derogation in terms of specific objectives, duration and reporting of the results.

The information provided in the Notes of Guidance relates to the assessment of cosmetic ingredients and final products from general chemical and microbiological safety points of view. However, safety assessment of chemical substances in certain physicochemical forms may need additional specific considerations, for example, the use of nanomaterials in cosmetics (see Section 3-8).

Other measures to safeguard the consumer's 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 (EC) No 1223/2009. 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.

[1] https://echa.europa.eu/documents/10162/13628/reach_cosmetics_factsheet_en.pdf

[2] "Workers" in this context are to be understood as persons who are actively involved in a particular activity of a production or manufacturing site where they may be exposed directly or indirectly to chemical substances. On the other hand, professional users who use the cosmetic products as part of their professional activity (e.g. hairdressers) and consumers shall not be considered as "workers".

In Regulation (EC) No 1223/2009 the term 'end user' means either a consumer or professional using the cosmetic product (Article 2, Definitions 1. (f)).

A number of definitions are listed in the Regulation (EC) No 1223/2009 (Art. 2) such as "cosmetic product", "substance", "(serious) undesirable effects", "nanomaterials" etc. (see Glossary). The important definition of "responsible person" is included (Art. 4), being *a legal or natural person established within the Community* (i.e. the manufacturer, importer or distributor). According to the above Regulation (Art. 4) *only cosmetic products for which a legal or natural person is designated within the Community as the "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):

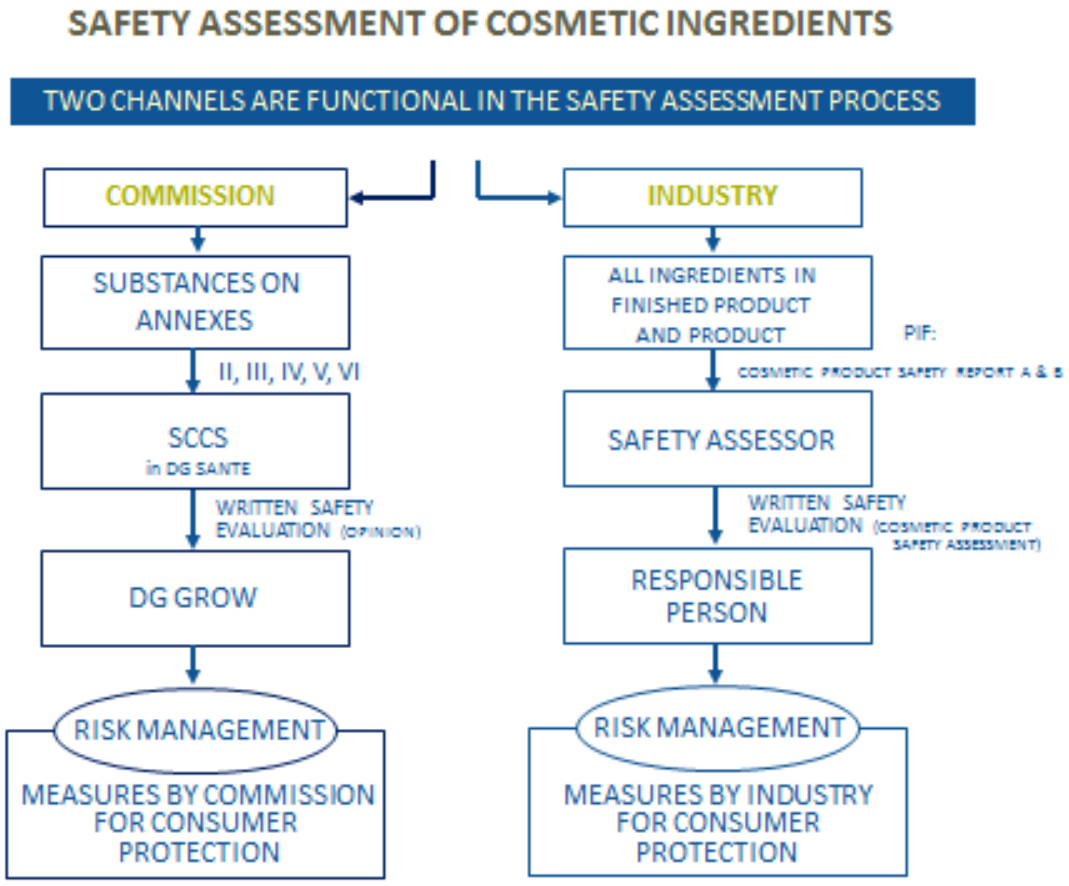

Fig. 1: Safety evaluation of cosmetic ingredients in the EU.

Primarily the substances in Annexes II, III, IV, V and VI fall under the responsibility of the SCCS (left part of Fig. 1). All ingredients of cosmetic products are the responsibility of the "responsible person", as defined by the Regulation (EC) No 1223/2009, through the safety assessor (right part of Fig. 1).

In general, the **safety evaluation** of cosmetic ingredients by the SCCS is based upon the principles and practice of the risk assessment process usually applied for chemical substances in the EU.

This risk assessment procedure is subdivided in 4 parts:

1) **Hazard identification** is carried out to identify the intrinsic toxicological properties of the substance, i.e. whether it has the potential to damage human health. It is based on the results of *in vivo* tests, *in vitro* tests, clinical studies, case reports, epidemiological studies and *in silico* methods. Intrinsic physical and chemical properties of the substance under consideration are also taken into account.

2) **Dose-response assessment:** In this part, the relationship between the exposure and the toxic response is evaluated. In the case of an effect with a threshold, usually the highest dose at which no adverse effects are observed (NOAEL) is determined. A dose without any effect may also be observed (NOEL). If the NOAEL cannot be derived, the lowest dose at which an adverse effect is observed (LOAEL) may be used. The Benchmark Dose (BMD) may be used as an alternative for the NOAEL, NOEL or LOAEL value. For details of the BMD approach, see Sections 3-4.5, 3-12.1. In the case of non-threshold carcinogens, the BMD or the T25 is used as a dose-descriptor (EFSA 2005, EFSA 2009; Dybing *et al.*, 1997).

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

4) **Risk characterisation:** In the case of a threshold effect, the **Margin of Safety (MoS)** is calculated by use of the following equation. Whereas the **NOAEL** is a dose descriptor for an external dose, the **$NOAEL_{sys}$** is a dose descriptor for the systemic exposure to a substance and is calculated from the NOAEL by use of the proportion of the substance systemically absorbed. **SED** represents the Systemic Exposure Dose. See Section 3-12.1 for details.

$$MoS = \frac{NOAEL_{sys}}{SED}$$

For non-threshold effects (*e.g.* a non-threshold carcinogenic effect), the lifetime risk is usually based on the T25 as described above. Alternatively, the Margin of Exposure (MoE) approach, for instance based on the BMD approach, can be used.

The assessment of carcinogens is described in Section 3-12.4.

The guidance provided in this document, in principle, equally applies to the safety assessments carried out by the SCCS and by the safety assessors of the cosmetic industry.

Risk characterisation is followed by **risk management** and **risk communication**, which are not the tasks of the SCCS, but of the European Commission (Fig. 1).

Besides the normal procedure when the industry submits a complete dossier, in some cases, either upon request of the Commission or on a voluntary basis, industry provides additional data on cosmetic ingredients that have been assessed in the past. An evaluation exclusively based on additional reports, together with summaries of earlier submissions, however, may not be adequate. Therefore, complete dossiers may be required case by case, even though a re-evaluation of only a part of a dossier appears necessary. Dossiers and full studies should be submitted in common formats such as pdf or Word. Only readable and searchable formats allowing copy/paste actions are accepted. Scanned documents that are not readable/searchable will not be accepted.

It is beyond the scope of the "Notes of Guidance" to discuss the whole process of risk assessment. Numerous review articles and text books exist on this topic. 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 evaluation, *e.g.* physical and chemical data, results of relevant toxicity studies, etc.

The contact point for dossier submissions and regulatory/risk management questions is: GROW-COSMETICS-AND-MEDICAL-DEVICES@ec.europa.eu

The SCCS address for scientific requests is: SANTE-C2-SCCS@ec.europa.eu

3-3 CHEMICAL AND PHYSICAL SPECIFICATIONS OF COSMETIC INGREDIENTS, FUNCTIONS AND USES

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 require physical and chemical property values as inputs for *in silico* estimation of properties and potential biological effects.

The basic and minimal specifications for any cosmetic ingredient to be evaluated are:

1) Chemical identity;
2) Physical form;
3) Molecular weight;
4) Characterisation and purity of the chemical including isomer composition;
5) Characterisation of the impurities or accompanying contaminants;
6) Solubility;
7) Partition coefficient (Log P_{ow});
8) Relevant physical and chemical specifications;
9) Homogeneity and stability.

For nanomaterials, special requirements for provision of physicochemical data apply (see Section 3-8). 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 presented. This is in order to provide full characterisation of the test chemical employed to generate the data in the dossier that the SCCS will consider.
Preference is clearly given to measured parameters of relevant batches compared to calculated values (*e.g.* log P_{ow}) or literature data (where often different batches are tested, with different impurity profiles).

In the following section, 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) number of the chemical, the

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

International Nomenclature of Cosmetic Ingredients (INCI) name or Common Ingredient Nomenclature (CIN, as in Regulation (EC) No 1223/2009) name and the EC number (see **Appendix 1** for more details) 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, (bio)technological or microbiological steps) and the materials used in their preparation to enable assessment of the probable structure and activity of the compound(s).

For the safety evaluation of a natural substance (*e.g.* an extract), complete information should be provided on the origin of the raw material (*e.g.* part of a plant), extraction method and any additional processes and/or purification steps used (see Section 3-9).

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 a 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. Any deviations will need to be justified.

3-3.2 Physical form

A description of the physical form should be given: powder, paste, gel, liquid. For nanoparticles, further information as specified in Section 3-8 should be given, including the particle size and its distribution.
For polymer ingredients, molecular weight distribution should be provided.

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 Identification and purity of the chemical and isomer composition

The experimental conditions of the techniques used for the chemical characterisation (UV, IR and NMR spectroscopy, Mass Spectrometry, chromatographic techniques, elemental analysis, etc.) as well as the resulting spectra, chromatograms etc. should be provided.

The substance(s) used in physical and chemical tests, toxicity studies, etc., mentioned in the dossier, must be either exactly the same material(s) under consideration or justifiably representative of the substances present in commercial products.
When a substance is a mixture of isomers, only the relevant isomer(s) used as a cosmetic ingredient should be included in the safety assessment. The other isomer(s) is/are considered as an impurity or impurities. Information on isomer composition should be provided.

The degree of purity must be clearly defined. The validity of the analytical methodology used must be shown. When a reference material/standard is used for the determination of purity, a certificate of analysis of the reference standard should be submitted. Purity of the active substance based on HPLC peak area can only be accepted when, 1) a reference material of known purity is used, 2) the HPLC recovery of the test material is known (and it should preferably be >98%), 3) the UV detection of the active substance is

performed at a specific wavelength (λmax), and 4) peak purity of the active substance is documented to be >99%.

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 impurities that may be present must be stated, along with their concentrations. Impurities should be characterised and quantified by an appropriate analytical method, *e.g.* by HPLC-PDA, LC-MS/GC-MS, NMR spectroscopy etc., using reference standards where appropriate. There is no specific recommendation available to assess the limit of acceptable non-CMRs impurities for cosmetic products.
Small changes in the nature of some impurities may 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 profile. The scientific validity of tests performed on batches of the substance with diverging purities deserves careful interpretation. Therefore, the responsible person must ensure that neither other impurities nor an increased level of impurities are present in the representative commercial material. For this, the stability of the synthesis process, including purification measures, is important. A change in these processes needs careful re-evaluation of impurities, even if the same level of purity is achieved.

3-3.6 Relevant physicochemical specifications

A typical physicochemical 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 and pressure), 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-filters, this spectrum is indispensable
- For nanomaterials and nanoparticles special requirements apply (see Section 3-8).

3-3.7 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 or other solvents.
When the solubility of the active substance in water is low (according to EU Method A.6), LC/MS should also be used to document the solubility and to rule out that the soluble material may be an impurity(or impurities) in the test material.
In general, solubility of substances poorly soluble in various solvents should be documented by LC/MS or another sensitive technique.
When the solubility of the active substance in HPLC mobile phase is low, LC-MS should be used for the detection and quantification of the active substance.

3-3.8 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 used for estimation should be specified.
The P_{ow} often 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.

3-3.9 Homogeneity and stability

Homogeneity data 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 and under conditions of use should be reported. In addition, the stability of the test substance under storage conditions as well as in typical cosmetic formulations should also be provided.

3-3.10 Functions and uses

For cosmetic substances under evaluation, the 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 consumer exposure via inhalation is possible and this should also be taken into consideration in the overall risk assessment.
In addition, other uses (*e.g.* consumer products, industrial products) and, wherever possible, the concentrations involved should be described.

3-4 RELEVANT TOXICOLOGICAL STUDIES ON COSMETIC INGREDIENTS

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 also the first step in the overall safety evaluation of cosmetic substances.
Traditionally, toxicological data relevant for humans have been obtained by investigating the toxicological profiles of the substances under consideration in experimental animals, using whenever possible the same exposure route as in humans (topical, oral or inhalation route).
Cosmetics and their ingredients, however, are excepted as animal testing has not been allowed since 11 March 2013 for any toxicological endpoint due to a strict testing and marketing ban for cosmetic ingredients taken up in the EU Cosmetic legislation (Regulation 1223/2009). For these products and their ingredients, validated alternative methods have to be applied to evaluate their safety. A variety of validated *in vitro* methods have been developed, mainly for genotoxicity and local toxicity.

Guidance on how to comply with the testing bans has been given by the Commission (COM/2013/135)(see Section 3-1 and **Appendix 3**). A factsheet has also been recently published by ECHA with respect to the interface between REACH and Cosmetics Regulations (see Section 3-1).

Toxicological studies required for safety evaluation usually cover acute toxicity, local toxicity and repeated dose toxicity as well as toxicokinetics.
Acute toxicity testing: animal studies performed to assess adverse effects which may result from a single exposure, usually carried out with high doses of the test substance, allow determination or estimation of a range of severe acute toxic effects including mortality (see Section 3-4.2).
Local toxicity: covering adverse effects on skin and eyes, often using high concentrations and single exposure.

The data from acute and local toxicity testing are mainly obtained for classification and labelling purposes (see the Regulation on the Classification, Labelling and Packaging of Substances and Mixtures (CLP) issued in 2008 (2008/1272/EC)).
Repeated dose toxicity: studies, usually performed with lower concentrations and involving daily administration/exposure for a prolonged period of time (*e.g.* 28 days/90 days/chronic, i.e. 1 year or longer; in certain cases, also studies on reproductive toxicity) allow for the determination of the NOAEL, LOAEL and BMD which are used in risk characterisation. These studies are also designed to identify target organs and may give an indication of mechanisms of action, etc.
Carcinogenicity studies are usually performed with mice and rats for a period of 18 to 24 months.

One of the obligations within the EU's regulatory framework is the development and validation of 3R-alternative methods that can provide an equivalent level of information on safety as the current animal tests but which use fewer animals, cause less suffering, or avoid the use of animals completely in scientific procedures (3R-strategy of Refinement, Reduction and Replacement).
In this respect, some refinement and reduction improvements have been made to the existing guidelines based on *in vivo* methodology. Moreover, a number of validated replacement guidelines based on *in vitro* methods have been developed. Regulatorily accepted replacement methods exist in the field of skin corrosion, skin irritation, mutagenicity/genotoxicity, phototoxicity, serious eye damage and dermal absorption. For eye irritation and carcinogenicity (Cell Transformation Assay, CTA), work is in progress. However, due to a variety of reasons, including the complexity of the mammalian *in vivo* systems, there are presently no validated (animal-free) replacement methods for acute and repeated dose toxicity, including reproductive and developmental toxicity, and carcinogenicity. There are also no relevant proposals currently ready in these areas for pre-validation/validation (Adler *et al.* 2011, JRC 2014a).

The European cosmetic legislation prohibits the marketing of finished products containing ingredients or combinations of ingredients that have been subject to animal testing after 2013 in order to meet the requirements of Regulation 1223/2009/EC. In view of the EU ban on the use of animal testing for cosmetic ingredients/products, and obligations to the 3Rs principle under different regulatory frameworks, the safety data for cosmetics needs to be derived from alternative non-animal means. Therefore, the SCCS and its predecessors have closely followed 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, memoranda on this particular subject have been issued on a regular basis (SCCNFP/0103/99, SCCNFP/0546/02, SCCP/1111/07, SCCS/1294/10). In addition to validated alternative methods, the SCCS may also accept, on a case-by-case basis, methods that are scientifically valid as well as new tools (*e.g.* "-omics" technology) 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 they have a sufficient amount of experimental data proving their relevance and reliability including positive and negative controls.
According to the Cosmetics Regulation, the experimental studies have 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 should be explained and scientifically justified (SCCNFP/0633/02).

This section describes animal tests (used for chemicals in general) and/or their existing validated 3R alternatives. Each test 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/timeframe of the in-life experiment is stated. In practice this implies that actual experimental work during the in-life phase must have been completed before 11 March 2013. The results, however, may be analysed afterwards. The date/timeframe of the in-life experiment may not only explain certain shortcomings in the studies when performed,

e.g. before the existence of the present testing guideline, but may also be used to identify whether the animal study had been performed before or after the date of the animal testing ban according to the Cosmetics Regulation. For the use of any animal studies for the safety assessment of cosmetic ingredients, see Section 3-1 and the scheme in **Appendix 3**.

The 3Rs alternatives comprise *in chemico/in silico* methods, *in vitro* methods and increasing use of combinations thereof, to obtain a sufficient evidence to allow reliable assessment of safety. Up to now only *in vitro* methods have been validated as predictive tools for local toxicity and mutagenicity/genotoxicity. It is generally acknowledged that before any testing (*in vitro/in vivo*) is carried out in the context of risk assessment, all possible information on the substance under consideration should be gathered from different available means. In this regard, *in silico* methodologies have gained importance. Several *in silico* methods are now available that cover different toxicological endpoints (*e.g.* genotoxicity, skin sensitisation). The predictive computational models are based on either (quantitative) structure-activity relationship ((Q)SAR), expert systems (rule-based models), or grouping/read-across from experimental data on analogous chemicals. Besides guidance documents on grouping/read-across (OECD 2014a), the OECD QSAR Tool Box[1] may be used for a systematic approach to the formation of chemical categories and other chemical analogies and predicting toxicological effects (OECD 2009a). The use of a combination of different approaches in an *in silico* battery usually increases confidence of the derived predictions. However, regardless of the *in silico* models used, the compounds under consideration should fall within the applicability domain of the respective model. Despite such developments, a recent report from the International Cooperation on Cosmetics Regulation (ICCR, 2014) concluded that the current use of *in silico* approaches is largely limited to internal decision making both at the industry and at the regulatory levels in most ICCR jurisdictions, and has not yet been fully adopted as a mainstream alternative to other testing methods for the safety assessment of cosmetic ingredients. Whilst recognising the need for appropriate choice of *in silico* tools and the expertise required for the use and interpretation of the results, and acknowledging certain limitations of the methods, the SCCS is of the opinion that *in silico* methodologies may be best used in a weight of evidence (WoE) approach to the risk assessment of a compound under consideration. This implies that for all the methodologies described in this section, *in chemico* (i.e. grouping and other chemical analogy approaches) and *in silico* (i.e. QSAR) methods should be applied, whenever possible, to derive estimates on toxicity before any experimental testing is considered.

Moreover, much effort is directed to the improvement and validation of other alternative methods and method combinations for the prediction of toxic effects.

The **Adverse Outcome Pathway (AOP)** is an approach which provides a framework to collect, organise and evaluate relevant information on chemical, biological and toxicological effects of chemicals in support of **Integrated Approaches to Testing and Assessment (IATA)** (OECD 2012a, 2012b, 2014b; Tollefsen *et al.*, 2014). The AOP framework has been taken up by the OECD to support harmonised collection, organisation and evaluation of relevant chemical, biological and toxicological information for use in human health and environmental risk assessment. The framework provides a tool for a knowledge-based safety assessment that relies on understanding toxicity mechanisms and helps to identify where methods should be developed and prioritised for validation and how the different approaches should be best integrated to ultimately replace the traditional animal tests. An OECD guidance document provides support in relation to which pieces of information are necessary to identify and document an AOP and how to present them. It also provides initial assistance on how to undertake the assessment of an AOP in terms of its relevance and adequacy (OECD 2012a).The AOP concept has been successfully applied to a number of human-relevant toxicological endpoints including skin sensitisation (OECD 2012b) (see Section 3-4.4), however the quantitative aspect is still a weak point.

[1] http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm

3-4.1 Toxicokinetics (ADME)

The term "toxicokinetics" is used to describe the time-dependent fate of a substance within the body. This includes absorption, distribution, metabolism and excretion (ADME). All of these processes need to be known in order to obtain a complete picture of how and to what extent compounds are handled by the body. The term "toxicodynamics" means the process of interaction of chemical substances with target sites and the subsequent reactions leading to adverse effects.

The testing guidelines for toxicokinetics including dermal absorption (EC B.36, 44, 45; OECD 417, 427, 428) 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 absorption of a substance under consideration, its metabolic transformation and fate can have an important effect on its distribution in the body and its excretion as well as toxic potential. Therefore, in specific cases, *in vivo* or *in vitro* biotransformation studies are required to prove or to exclude certain adverse effects. However, the conduct and use of such animal studies is restricted due to the animal testing ban for cosmetic ingredients in the EU (see Section 3-1). Conducting new *in vivo* animal studies on toxicokinetics is no longer an option for cosmetic ingredients in the European context, as the deadline of the animal testing ban of 11 March 2013 has passed. For the use of any *in vivo* studies for the safety assessment of cosmetic ingredients, see Section 3-1 and the scheme in **Appendix 3**.

Although toxicokinetic data for cosmetic ingredients are only available in certain circumstances, their relevance may be high for extrapolating both *in vivo* and *in vitro* animal data to the human situation.

Any route-to-route extrapolation can be performed in a case-by-case manner based on expert judgement of scientific information, including available toxicokinetic information. It can, however, only be performed in the case of systemic toxicity. In this regard, not only the degree of absorption, but also metabolism should be considered (ECHA, 2012a, 2015). See for example the oral to inhalation extrapolation in Section 3-12.1.

An in-depth review of the current status of toxicokinetics in the risk assessment of cosmetics and their ingredients can be found in JRC reports (Adler *et al.* 2011, JRC Scientific and Policy Report 2013a, 2014a,b, 2015a).

At present, no validated alternative methods that completely cover the field of ADME exist. Some *in vitro* models are suitable for contributing to the assessment of the absorption of substances from the gastro-intestinal tract (*e.g.* Caco-2 cell cultures) or the biotransformation of substances (*e.g.* isolated hepatocytes, Hepa RG™ cells, and their cultures), but most of the many existing models have not been fully validated yet (Adler *et al.*, 2011; Eskes *et al.*, 2005; JRC Scientific and Policy Report 2013a, 2014a, 2014b, 2015a).

Although not officially recognised 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. Given 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 standardised 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 and similar models indeed have a number of advantages and disadvantages (Grès *et al.*, 1998; Le Ferrec *et al.*, 2001; Thomas *et al.*, 2008; Adler *et al.*, 2011). Great attention is particularly required in cases where 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). The European Union Reference Laboratory for Alternatives to Animal Testing (EURL-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 a limited number of cases, human toxicokinetic study results were available to the SCCS for cosmetic ingredients, *e.g.* p-phenylenediamine (SCCP/0989/06, SCCS/1443/11), 4-methyl benzylidene camphor (SCCP/1184/08), n-butylparaben (SCCS/1446/11, SCCS/1348/10), zinc pyrithione (SCCS/1512/13). For further examples see Section 3-4.1.2. It would be a step forward to include more human toxicokinetic studies for Annex cosmetic ingredients provided that a) risk assessment cannot adequately be performed by use of other data/methodologies and that b) such human studies are ethically acceptable.

3-4.1.1 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, of which the rate-determining layer is considered to be the *stratum corneum*. A number of factors play a key role in this process, including molecular weight, charge, lipophilicity of the compounds, the thickness and composition of the *stratum corneum* (which depends on the body site), the duration of exposure, the amount of topically applied product, the concentration of target compounds, occlusion, vehicle, etc.

Dermal/percutaneous absorption has been described by several international bodies (ECETOC 1993, US EPA 1996a, OECD 2004, WHO 2006, OECD 2011a) 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.

a. Guidelines for dermal/percutaneous absorption studies

Dermal/percutaneous absorption studies can be performed in principle *in vivo* or *in vitro*. Their testing protocols form part of the official EU and OECD test methods (EC B.44, 45; OECD 427, 428). Detailed guidance on their performance is also available (SCs/01/04, OECD 2004, 2011a). In addition, the SCCNFP adopted a first set of Basic Criteria for the *in vitro* assessment of dermal absorption of cosmetic ingredients in 1999 (SCCNFP/0167/99). The SCCS updated this Opinion in 2010 (SCCS/1358/10). A combination of the EU and OECD Guidelines with the SCCS "Basic Criteria" (SCCS/1358/10) is considered to be essential for performing appropriate *in vitro* dermal/percutaneous absorption studies for cosmetic ingredients.

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. These amounts can then be taken into consideration to calculate the margin of safety.

Numerous specific parameters or working conditions need to be taken into consideration:

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 including contact time. Several concentrations, including the highest concentration of the test substance in a typical formulation, should be tested.
8) Regular sampling is required during the whole exposure period, taking into account delayed penetration into skin layers.
9) Appropriate analytical techniques should be used. Their validity, sensitivity and detection limits should be documented in the report.
10) 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.
11) 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%.
12) 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.
13) When dermal absorption studies are performed, radioactive labelling of the substance under consideration is often used in order to increase 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.
14) 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).

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 the 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.

In addition, 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 the dermal absorption of certain ingredients. It is clear that in such formulations, in the absence of further specific studies, 50% bioavailability of a particular substance will have to be assumed. This conservative value may also be used in cases where no or inadequate absorption data are available.

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.* colourants or UV-filters 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). Adequate detection of substances poorly soluble in water is important in the receptor fluid of *in vitro* dermal absorption study to ascertain that the dermal absorption concerns the active substance and not the impurities. For nanomaterial, it is also important to ascertain whether the substance absorbed through the skin was in nanoparticle form or in a dissolved chemical state.

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 of the Notes of Guidance and/or very high variability, the mean + 2SD may be used as dermal absorption for the MoS calculation (see Section 3-12.2).

A retrospective study of the Annex (to Cosmetic Regulation) substances present in the opinions (2000-2014) of the SCCS and its predecessors has shown that the cosmetic ingredients characterised by the following physicochemical properties:

- MW>500 Da,
- High degree of ionisation,
- Log P_{ow} ≤-1 or ≥4
- Topological polar surface area >120 Å^2,
- Melting point > 200°C

may be indicative of low or very low dermal absorption. For dealing with data on low or very low dermal absorption, see Sections 3-5.1, 3-12.1 and 3-12.2

3-4.1.2. Metabolism

a) General aspects of metabolism of xenobiotic substances

Metabolism of xenobiotic substances in mammals mainly occurs via phase I and/or phase II reactions mediated by xenobiotic metabolising enzymes (XMEs). Phase I reactions such as oxidation, reduction, hydrolysis etc. introduce functional groups into the molecule (functionalisation). Phase II reactions render the xenobiotic substance or its metabolite(s) more hydrophilic and excretable via bile or urine, by conjugation mainly with glutathione,

glucuronic acid or sulfate. In most cases, phase I metabolites which may be reactive are also inactivated by these conjugation reactions.
Metabolism of xenobiotic substance may differ from species to species due to different protein structures and substrate specificities of XMEs and different levels of expression and regulation of the subclasses of XMEs (isoenzymes) as well. These potential species differences are in general considered in risk assessment by the use of an ***interspecies* default factor** for toxicokinetics including metabolism (see Section 3-12.1). However, the use of a fixed factor may under certain circumstances lead to errors in risk assessment if large interspecies differences of metabolism between laboratory animals and humans are not recognised and/or not adequately accounted for. Whereas such cases seem to be rare, some well-characterised substances have been described possessing different carcinogenic potencies based on different metabolism between laboratory species compared to humans (Oesch and Hengstler, 2014; Hengstler *et al.*; 1999).

In mammals, expression and regulation of XMEs have been shown to vary between strains and genders or due to other factors. Such differences of genetic or environmental nature are also known in humans. Individual factors may be gender or age dependent differences, for instance between young children, adolescents or adults of different age. Individual differences due to nutrition, health status (disease) or pregnancy may also play a role. These potential individual differences are considered in risk assessment by the use of an ***intraspecies* default factor** for toxicokinetics (including metabolism) (see Section 3-12.1). This intraspecies factor may need to be adapted if substance specific information is available (*e.g.* human XME polymorphisms).

In general, metabolic capacity of XMEs in mammalian liver is much higher than in extra-hepatic tissues including skin when based on per gram of tissue. In addition to quantitative differences in metabolic capacity there are also major differences in the equipment, constitutive expression and regulation of XMEs between mammalian liver and extra-hepatic tissues including skin (Gundert-Remy *et al.*, 2014; Oesch *et al.*, 2007; Oesch *et al.*, 2014). Therefore, in some cases, when an XME isoenzyme form is not active in liver such as human N-acetyltransferase 1 (NAT1), extrahepatic metabolism including skin may qualitatively differ from that in the liver (*e.g.* hair dyes p-Phenylenediamine (A7) SCCS/1443/11 and 6-Amino-m-cresol (A75) SCCS/1400/11).

Although data on systemic or dermal metabolism are not a regular requirement for risk assessment by the SCCS, such data are helpful and sometimes required to complete the mosaic of the toxicity profile of a cosmetic ingredient. Data on metabolism of a substance is primarily obtained by *in vitro* or *ex vivo* methods using cellular or tissue materials from laboratory animals. Increasingly, cells and cellular fractions or organ specimen from human sources are available, although limited. Much progress has been made during the last years in preserving metabolic capacity and regulation of XMEs in cells in culture, for instance by developing 3D cultivation techniques. At present, these methods are still under validation. EURL-ECVAM has carried out a multi-study validation project on human cytochrome P450 (CYP) induction in order to assess the reliability and relevance of two CYP induction *in vitro* methods. The cells involved were cryopreserved human HepaRG™ cells (cryoHepaRG) and cryopreserved human primary hepatocytes (cryoheps). This project will contribute to the building of an *in vitro* platform for assessing metabolism and toxicity (JRC 2014b).

Extrapolation from *in vitro* metabolism data to the *in vivo* situation of laboratory animals or humans may be difficult although some progress has been made, in particular in combination with PBPK modelling (Coecke *et al.*, 2013; Wilk-Zasadna *et al.*, 2014; see also Section 3-4.1.3). Often, *in vivo* data from laboratory animals is helpful or even indispensable in order to clarify if or to which extent relevant metabolites are formed (see OECD 417 on toxicokinetics). However, generation and use of animal *in vivo* data for cosmetic ingredients is restricted, due to the animal testing ban of the Cosmetics Regulation. For the use of *in vivo* data for risk assessment of cosmetic ingredients see Section 3-1 and **Appendix 3**.

Because of the species differences of XMEs, human *in vivo* data is the gold standard, however it should be considered as a last resort and with the restrictions mentioned in Section 3-1. Some examples including human toxicokinetic data can be found in several SCCS opinions such as for Parabens (SCCS/1348/10, SCCS/1514/13), Triclosan (SCCP/1192/08) and aromatic amines (hair dyes Toluene-2,5-diamine (A5) (SCCS/1479/12), p-Phenylenediamine (A7) (SCCS/1443/11), 6-Amino-m-cresol (A75) (SCCS/1400/11), SCCS/1400/11, Zinc pyrithione (SCCS/1512/13). In some of these human toxicokinetic studies with cosmetic ingredients after dermal exposure, high inter-individual differences in toxicokinetic parameters were observed (partly >10), potentially due to differences between slow and rapid metabolisers (p-Phenylenediamine (A7) SCCS/1443/11; Triclosan, SCCP/1192/08).

b) Metabolism in skin

Skin is both a physical and a biochemical barrier to the absorption of chemicals, micro-organisms and particulate materials. Besides the role of the *stratum corneum* as the most critical structure with barrier function, there is growing evidence that XMEs may have physiological functions in addition to defence of xenobiotic substances. Hence, constitutive expression and regulation (induction) of XMEs is tissue-specific, also in skin. Most of the major enzymes found in the liver may also be present in the skin but often at lower activity levels compared with other tissues. Phase II reactions in skin apparently play a greater role than phase I reactions, the metabolic capacity of which is considered very low. It is plausible to assume that the role of phase II enzymes in skin is primarily to inactivate exogenous substances, thus supporting the barrier function of skin (Gundert-Remy *et al.*, 2014; Oesch *et al.*, 2007; Oesch *et al.*, 2014; SCCP/1171/08).
There are examples that only small percentages of substances are metabolised in skin. On the other hand, in some cases nearly complete biotransformation during dermal absorption was observed. Whereas the fate of chemicals in the skin with regard to the type and degree of metabolism was considered a matter of uncertainty (SCCP/1171/08), much progress has been made in the characterisation of XMEs in human skin and cutaneous metabolism, including the metabolic competence of cutaneous cell types, such as keratinocytes and dendritic cells. Moreover, the development and metabolic characterisation of *in vitro* skin models has made progress. The comparison of XME activities of native human skin, 2D and 3D models (EpiDerm™ and SkinEthic™ reconstructed human epidermis (RhE) models) and monolayer cultures of HaCaT cells showed promising similarities (Hewitt *et al.*, 2013; Oesch *et al.*, 2014). However, additional work is necessary and none of these skin models has yet been validated for metabolism.

These skin models may help in the future to clarify important questions. For instance, oxidative bio-activation of prohaptens to haptens in the skin is considered an immunological hazard of topically applied xenobiotics. Data and reviews on prohaptens requiring metabolic activation in the skin are available (Bergström *et al.*, 2007; Karlberg *et al.*, 2008, SCCS/1459/11). Some data suggest that the risk of cutaneous allergy by p-Phenylenediamine (PPD, hair dye A7) may depend on the individual capability of inactivating PPD in skin by N-acetylation (NAT1), the slow metabolisers hence being at higher risk of PPD-induced allergy than the rapid metabolisers (Kawakubo *et al.*, 2000).

3.4.1.3 PBPK modelling

Physiologically based pharmacokinetic (PBPK) modelling is the mathematical description of pharmacokinetic (ADME) processes of substances in living organisms.
PBPK models are based on interrelationships among key physiological, biochemical and physicochemical determinants of ADME processes.
PBPK models can be used to refine risk assessments with respect to *e.g.* the following issues:

- Prediction of target tissue doses

- Intra- and interspecies extrapolation (variability issues)
- Route-to-route extrapolation
- Dose extrapolation
- Replacement of default assessment factors by more specific, substance-derived factors

Physiological, anatomical, biochemical and physicochemical parameters are necessary to build up PBPK models in which ADME processes are represented by equations and organs are represented by body compartments.
Whereas physiological and anatomical parameters are readily available, biochemical (*e.g.* metabolic rate constants) and physicochemical parameters (*e.g.* partition coefficients) are substance-specific and can be measured values or estimated values (the latter *e.g.* obtained by fitting processes using the PBKP model).
The use of estimated values in further modelling estimates might however increase uncertainties associated with a model.

Confidence in a model can be high if the following conditions are met:

- All physiological, anatomical and biochemical parameters used are biologically plausible
- Equations used are mathematically correct
- Measured values are used instead of estimated values
- Thorough sensitivity analysis (sensitivity of the system to parameter change) is available and documented
- In case of estimated values: when sensitivity analysis has documented that they do not significantly influence the model output
- The model reproduces experimental data which were not used to estimate the parameters

The current status and applicability of PBPK modelling has been recently reviewed (Bessems *et al.*, 2014; ECHA, 2014b; EFSA 2014).

When estimated data from PBPK models are submitted to SCCS which are intended to be used for quantitative risk assessment (i.e. MoS calculation), then it should also be demonstrated that a model correctly predicts experimental data that have not been used to build the model, preferably in the form of a peer-reviewed publication. Further, all equations, input parameters, information of software used should be provided – preferably in a tabular form.

SCCS will use data from PBPK models for quantitative risk assessment only if sufficient details are provided so that the calculations can be evaluated. Otherwise, the data may only be used as supporting information.

3-4.2 Acute toxicity

The term **acute toxicity** is used to describe the adverse effects, which may result from a single exposure (i.e. a single exposure or multiple exposures within 24 hours) to a substance. Exposure relates to the oral, dermal or inhalation routes (ECHA, 2015).

If data on acute toxicity *in vivo* are available, these data should be provided. However, in the light of the animal testing ban for cosmetic ingredients (see section 3-1 and **Appendix 3**), data on acute toxicity is not mandatory for assessing the safety of cosmetic ingredients for consumer uses but a weight of evidence approach may be sufficient - such as justified conclusions from chemical grouping/read-across, (Q)SAR, *in vitro* studies, or repeated dose toxicity studies.

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 with respect to acute toxicity (2008/1272/EC).

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

- The **fixed dose method** (EC B.1bis, 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) allows the determination of a range of exposure doses where lethality is expected. The test follows a complex stepwise dose scheme. 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. The guideline significantly reduces the number of animals used.

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.

3) Acute dermal toxicity

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

3-4.3 Corrosion and irritation

1) Skin corrosion and skin irritation

Skin corrosion is defined as *irreversible* damage to the skin, namely visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to 4 hours. Corrosive reactions are typified by ulcers, bleeding, bloody 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).
Dermal irritation is defined as the production of reversible damage of the skin following the application of a test substance for up to 4 hours (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 of chemicals 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. An example is potassium hydroxide. 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.

There is one *in vivo* test method to assess the potential of a substance to cause acute skin corrosion / dermal irritation (EC B.4, OECD 404). Skin corrosion and irritation data obtained from this test should be provided when available if the test was performed before the animal testing ban or if the data was obtained in order to be in compliance with other legislations, *e.g.* REACH.

For **skin corrosion** testing, at present, there are three test guidelines on *in vitro* replacement alternatives including six different validated test methods. The three test guidelines available are:

1) The Rat Skin Transcutaneous Electrical Resistance (TER) test which uses excised rat skin as a test system and its electrical resistance as an endpoint (EC B.40bis, OECD 430).

2) The Reconstructed Human Epidermis (RhE) Test Method which includes four validated commercialised human skin models i.e. EpiSkin™, EpiDerm™ SCT (EPI-200), SkinEthic™ RHE and epiCS® (former Epidermal skin test 1000). They all consist of reconstructed human epidermal equivalent and use cell viability as an endpoint (EC B.40bis, OECD 431). Only the EpiSkin™ and EpiDerm™ models are included in EC B.40bis.

3) The *In vitro* Membrane Barrier Test Method (OECD 435) currently only includes the commercially available Corrositex® test method and has not yet been adopted in the European legislation (cf. REACH).

For **skin irritation** testing, at present, there is one test guideline on *in vitro* replacement alternatives:

1) The Reconstructed Human Epidermis (RhE) Test Method (OECD 439), including four commercially available *in vitro* test methods which have been validated to be used as:
   - a stand-alone replacement test for *in vivo* skin irritation testing, or as
   - a partial replacement test, within a tiered testing strategy.

   The four commercially available *in vitro* methods are: EpiSkin™, EpiDerm™ SCT (EPI-200), SkinEthic™ RHE and LabCyte EPI-MODEL24SIT. Only the first three RhE models are included in EC B.46.
   Similar to TGs 430, 431 and 435, the revised TG 439 (July 2013) also includes accompanying skin irritation performance standards developed by EURL-ECVAM to facilitate the validation and assessment of possible future RhE-based test methods for the purpose of skin irritation testing. The endpoint used in the RhE test method is cell mediated reduction of MTT (3-(4,5)-dimethyl-2-thiazolyl-2,5-dimethyl-2H-tetrazolium bromide). To obtain better sensitivity, while maintaining similar specificity, a second endpoint has been suggested: interleukin-1α (IL-1α) production.

The *in vitro* test for skin irritation testing has been found useful by the SCCS for the testing of cosmetic ingredients. However, there are concerns about reducing substances, hair dyes and colorants since these can interfere with the formazan colour evaluation (Lelièvre *et al.* 2007, SCCS/1392/10). When these substances need to be tested, a different technique, involving HLPC separation prior to quantification, should be used (SCCS/1392/10). In this context, Cosmetics Europe evaluated the use of such a refined method for formazan quantification, applicable for all reconstructed human tissue methods (Alépée *et al.*, 2015). According to ESAC (ECVAM's Scientific Advisory Committee) recommendation, this HPLC technique can be used in test methods using reconstructed human tissues as an alternative to the measurement of absorbance by optical density (OD), for coloured and non-coloured test chemicals. Revised in July 2015, TGs 431 and 439 support the use of HPLC-spectrophotometry-based methods.

OECD has developed a Guidance document on an integrated approach on testing and assessment (IATA) for skin corrosion and irritation (OECD 2014b). The Guidance document has two aims: 1) to propose an integrated approach for replacing the strategy provided in the *in vivo* test guideline (OECD 404) and 2) to provide consistent information on key

performance characteristics of each of the individual information sources comprising the IATA, and to provide guidance for decision making within the approach.

2) Serious eye damage and eye irritation

Severe (serious) eye damage 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 (EC B.5, OECD 405).
Eye irritation is defined as the occurrence of changes in the eye following the application of a test substance to the anterior surface of the eye, which are fully reversible within 21 days of application (EC B.5, OECD 405).

There is one *in vivo* test method to assess the potential of a substance to cause acute serious eye damage / irritation (EC B.5, OECD 405). Serious eye damage and irritation data obtained from this test should be provided if available and if the test was performed before the animal testing ban or if data was obtained in order to be in compliance with other legislations, *e.g.* REACH.

For **serious eye damage testing and/or identification of chemicals not triggering classification for eye irritation or serious eye damage**, at present, there are five test guidelines adopted on *in vitro* alternatives:

a) Two of them are ***organotypic test methods***, making use of tissues obtained from slaughterhouses (OECD 2011b):

1) The **Bovine Cornea Opacity Permeability (BCOP) test method** measuring the ability of a test chemical to induce opacity and permeability in an isolated bovine cornea (EC B.47, OECD 437).

2) The **Isolated Chicken Eye (ICE) test method** evaluating the ability of a test chemical to induce toxicity in an enucleated chicken eye (EC B.48, OECD 438).
Recently, the International Association for Soaps, Detergents and Maintenance Products (A.I.S.E.) proposed histopathological evaluations as an additional endpoint for ICE to evaluate some specific products i.e. detergents and cleaning products (Cazelle *et al.*, 2014 & 2015).

Both the BCOP and ICE test methods are able to identify:

(i) chemicals inducing serious eye damage (Cat. 1 according to UN GHS definitions) and
(ii) chemicals not requiring classification for eye irritation or serious eye damage. (No Category according to UN GHS definitions)

Two other organotypic assays, i.e. the **Isolated Rabbit Eye** and **Hen's Egg Test-Chorio Allantoic Membrane (HET-CAM)** have been developed but not implemented as an OECD guideline. However, they may provide supportive evidence to identify serious eye damage (JRC website 2014).

b) In addition to the organotypic test methods, a set of ***cytotoxicity and cell function-based in vitro tests*** are also available:

3) The **Short Time Exposure (STE) test method** uses a rabbit corneal cell line to evaluate the eye irritation potential of a chemical by measuring its cytotoxic effect (OECD 491). The STE test method can be used to identify chemicals inducing serious eye damage (Cat. 1) and chemicals not requiring classification for eye irritation or

serious eye damage. The STE test has, however, limitations with respect to highly volatile chemicals and solid chemicals other than surfactants.

4) The **Fluorescein Leakage (FL) test** measures the toxic effects after a short exposure time of the test substance by an increase in permeability of sodium fluorescein through the epithelial monolayer of MDCK kidney cells cultured on permeable inserts (OECD 460). The FL test is recommended as part of a tiered testing strategy for regulatory classification and labelling of severe eye irritants (Cat. 1), but only for limited types of chemicals (*i.e.* water soluble substances and mixtures; strong acids and bases, cell fixatives and highly volatile chemicals have to be excluded).

The **Cytosensor Microphysiometer (CM) test method** has been validated by ECVAM in 2009, is performed on a sub-confluent monolayer of adherent mouse L929 fibroblasts cultured in a sensor chamber using a pH-meter to detect changes in acidity. A draft OECD TG on the use of this method as part of a tiered testing strategy for identifying ocular corrosive and severe irritant chemicals (Cat. 1) and chemicals not triggering a classification for eye irritation has not yet been approved. The CM test method cannot exclude mild eye irritant potential and only applies for water soluble chemicals (substances and mixtures) as well as non-water soluble solid, viscous chemicals or suspensions that maintain uniformity during analysis time. This methodology has in particular been used in the USA.

In addition, the neutral red release, and the fluorescein leakage and red blood cell haemolysis test also underwent retrospective validation and peer review by ESAC (ESAC 2009b).

c) Finally, ***Reconstructed human tissue (RhT)-based test methods*** available include:

5) The **Reconstructed Human Cornea-like Epithelium (RhCE) test method** (OECD 492), which evaluates the ability of a test chemical to induce cytotoxicity via the MTT assay. The recently adopted TG includes the HPLC/UPLC technique for measuring the formazan formation, especially important for the evaluation of chemicals which may interfere with MTT-formazan measurement by direct reduction of MTT or colour interference. To date, RhCE models can be used as *in vitro* methods to identify chemicals not requiring classification and labelling for eye irritation or serious eye damage. Consequently, these models are not suitable for determining the potency of eye irritancy. At present, only the **EpiOcular™ EIT**[1], using a commercially available non-transformed human-derived epidermal keratinocyte model, is covered by this TG.

Currently, the available replacement alternatives for serious eye damage and eye irritation testing cannot identify any mild eye irritancy potential.

For **eye irritation** testing, at present, **there is no validated alternative method** fully replacing the *in vivo* test (OECD 405, EC B.5). This test has been subject to refinement and reduction measures. It was also indicated in the last update of OECD 405 that histopathology is an additional endpoint in ocular safety testing.
Neither a single *in vitro* assay nor a testing battery has been validated as a stand-alone replacement for the *in vivo* test. 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

[1] EIT- Eye Irritation Test

data may lead to a classification as irritant or non-irritant. It is noted that the existing *in vitro* models may fail to discriminate non-irritants from 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 notes that, in the above tiered approach, safety testing for eye irritation using human volunteers 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. 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 differentiate between non-irritants from severe irritants, leaving all others to the (mild/moderate) irritant categories.

3-4.4 Skin sensitisation

A skin sensitiser is an agent that is able to induce specific immunological reactivity after contact with the skin and penetration into the epidermis. 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 fully validated and complete *in vitro* test methodology 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, EC B.42) uses an inbred strain of mice and is based on the extent of stimulation of proliferation of lymphocytes in regional lymph nodes draining the site of application of the test substance. It is an objective method giving the result as a stimulation index, 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. For the LLNA also ISO guideline 2002 (ISO, 2002) and an updated ISO guideline (ISO, 2010) exist.

The **reduced LLNA (rLLNA)** has been added as an option in the amended OECD TG429 in 2010. This is a reduced version of the LLNA, using only a negative control group and the equivalent of the high-dose group from the full LLNA. The rLLNA does not allow the determination of the potency of a sensitising chemical as only one dose is tested. When compared with the full LLNA, the rLLNA may produce a few false negatives (1-2% in the reference document).
The OECD mentions the possibility of performing a rLLNA, but with certain restrictions and certainly not when dose-response information is required (OECD 429, EC B.42).
Simultaneously, work at the OECD level took place accepting the LLNA using non-radioactive methodologies, *e.g.*:

- 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 Enzyme-Linked Immunosorbent Assay (ELISA) BrdU (5-bromo-2-deoxy-uridine), which is a 2nd generation ELISA with colorimetric or chemiluminescent detection that quantifies the DNA synthesis within the lymph node cells (EC B.51, OECD 442B).

The LLNA is an alternative method used on mice that refines the methodology in comparison with the traditional guinea pig-based models, which are described in the following.

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.

As far as replacement of *in vivo* tests for skin sensitisation is concerned, two non-animal test methods have been accepted by the OECD. They should not be used as stand-alone tests. Instead, these tests should be included in Integrated Approaches for Testing and Assessment (IATA) according to the OECD. These assays and further advances in the field are based upon the current level of knowledge on the mechanism of skin sensitisation/allergic contact dermatitis, more specifically on the five key mechanistic pathways of skin sensitisation. These consist of (i) haptenation (mostly covalent binding of a chemical sensitiser to skin protein), (ii) epidermal inflammation (release of pro-inflammatory signals by epidermal keratinocytes), (iii) dendritic cell activation, (iv) dendritic cell migration (movement of hapten-peptide complex bearing dendritic cells from skin to draining lymph node), and (v) T-cell proliferation. *In vitro* tests are being developed that are representative for these steps.

This mechanistic knowledge has been clustered in a so-called AOP approach (see introductory part of Section 3-4) describing the sequence of key events starting from the molecular initiating event to the adverse outcome, allergic contact dermatitis, and considered to be the way forward in the general field of alternatives to develop new *in vitro* tests (OECD 2012a; Vinken *et al.*, 2013). The AOP concept has been successfully applied to skin sensitisation (OECD 2012b). The molecular initiating event (MIE) in this AOP is the covalent binding of the substance to protein.

A test to assess peptide reactivity is the "***in chemico***" **skin sensitisation Direct Peptide Reactivity Assay (DPRA)** (OECD 442C). 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). DPRA is a transferable test method and sufficiently reproducible within and between laboratories. The test method, however, is not proposed as a stand-alone full replacement since DPRA is covering only one single biological step in the skin sensitisation pathway and it does not consider metabolic capacity. DPRA information may also have the potential to contribute to potency assessment. However, additional work is still required to determine how DPRA results can be exploited within integrated approaches for potency prediction using preferably human data.

Another method in the same field that has been adopted by OECD is the ***in vitro*** **skin sensitisation ARE-Nrf2 luciferase test** (OECD 442D), which measures activation of keratinocytes and determines the 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 sensitisers (Natsch, 2010). Currently, the only *in vitro* ARE-Nrf2 luciferase test method covered by this test guideline is the KeratinoSens™ test method.
Concentration-response information, generated with the KeratinoSens™, may play a role in integrated approaches for potency prediction, but the test guideline cannot be used on its own to predict potency for safety assessment decisions. The assay also seems to have a great potential for mixtures testing. It has been reported that the KeratinoSens™ assay indicates high sensitivity in detecting minor components with sensitising potential (Andres *et al.*, 2013).
Given the fact that the test method addresses only one single biological step in the overall mechanism of skin sensitisation and considering its known limitations such as the limited consideration of metabolic aspects and the ability to detect only cysteine-reactive chemicals, it has been recommended that the method should only be used in combination with other information sources.

A further key event in the AOP is dendritic cell activation. Also for this step an *in vitro* test will become available in the near future. Indeed, a **human cell line activation test (h-CLAT)**, based on the enhancement of CD86 and/or CD54 expression in THP-1 cells, has passed evaluation by EURL-ECVAM (JRC, 2015b) and is currently being evaluated by the OECD to be accepted to their test guideline programme. The **U-SENS™ Test** (former **MUSST**) is based on the same principle and uses the cell line U-937 human lymphoma. This assay was validated in an industry-led study and following positive evaluation by EURL-ECVAM of the submission it will soon enter the ESAC peer review process.

An extensive review of the status of *in vitro* testing in this field can also be found in a JRC report (Adler *et al.*, 2011; JRC 2014) and in a recent publication (Reisinger *et al.*, 2015).

The DPRA and KeratinoSens™ test methods are proposed in the Test Guidelines to be used for supporting the discrimination between sensitisers and non-sensitisers within IATA (see introductory part of Section 3-4). Both methods can indeed be used in a Weight of Evidence (WoE) approach in an *in vitro* test battery for the assessment of skin sensitisation.

There are other new developments in the area of skin sensitisation, however, these are as yet less developed and in different stages of validation. In the future these could eventually become of importance. Among these (not exclusively) are assays measuring peptide binding (assay 1), keratinocyte activation (assays 2-7) or dendritic cell activation (assays 8-10). The development of standardised assays that measure T cell activation *in vitro* is not as advanced yet.

1) **Peroxidase Peptide Reactivity Assay (PPRA)**, which encompasses an enzymatic activation step based on horseradish peroxidase (HRP)/hydrogen peroxide to the peptide reactivity assay to detect pro-haptens (Gerberick *et al.*, 2009 and Troutman *et al.*, 2011)
2) **LuSens**, a new stable ARE reporter gene assay based on a human keratinocyte cell line for the identification of skin sensitisers. The assay provides information on both protein reactivity and keratinocyte activation. The LuSens assay utilises a similar principle as the KeratinoSens™ assay: human keratinocytes harbouring the luciferase reporter gene under the control of an antioxidant response element (ARE) are used to assess the induction of the cytoprotective responses elicited by the genes controlled by the ARE. The luciferase activity is used as a measure for this response (Ramirez *et al.*, 2014): EURL-ECVAM positively assessed the information generated in phase of LuSens validation process and will progress the submission into ESAC peer review.
3) **IL-8 Luc Assay** was evaluated in a validation study coordinated by JaCVAM in Japan and is currently under peer review by the same organisation. The development of a test guideline for this method will be included in the OECD work programme of 2015. The IL-8 Luc assay assesses the effects of chemicals on IL-8 promoter activity,

evaluated using THP-1 cells transfected with the IL-8 luciferase reporter gene (Takahashi *et al.*, 2011).

4) **The Reconstituted Human Epidermis (RhE) IL18 Potency Test** uses two readouts: intracellular IL-18 release is used to discriminate between sensitisers and non-sensitisers, and viability as a measure of sensitising potency (Gibbs *et al.*, 2013).
5) **The SENS-IS** is a gene expression-based test method proposed to discriminate between sensitisers, non-sensitisers and irritants by analysing the expression of a panel of 65 genes grouped in one gene set for irritancy and two (SENS-IS and ARE) for sensitisation. A test substance is classified as sensitiser on the basis of the number of overexpressed genes (compared to the solvent control) measured by qRT-PCR in Episkin tissues (SkinEthic, France). In addition, the test method allows the classification of sensitisers into potency categories on the basis of the concentration of chemical needed to induce a positive response.
6) Currently, also modifications of already existing skin models used for skin irritation have been reported. For instance development of a new *in vitro* skin sensitisation assay: **Epidermal Sensitisation Assay- EpiSensA** is used for detecting skin sensitisers by measuring the expression of ATF3, DNAJB4, and GCLM genes. This assay correctly predicted the 16 reference chemicals recommended by ECVAM including pre-/pro-haptens. It is currently under further evaluation with an increased number of chemicals. EpiSensA, as all the methods, uses a reconstituted human epidermis
7) Another modified assay is the **Peripheral Blood Monocyte Derived dendritic Cells (PBMDC)** test based on flow cytometric measurement of CD86 expression as an activation marker for sensitisation process. The inter-laboratory performance of this test was considered promising (Reuter *et al.*, 2015).
8) **The Genomic Allergen Rapid Detection (GARD)** test method is a transcriptomics-based *in vitro* assay proposed to discriminate between skin sensitising and non-sensitising chemicals using gene expression as a read-out. The cell line used is the human myeloid leukemia-derived cell line MUTZ-3, as a surrogate model for *in vivo* dendritic cells (Johansson *et al.*, 2011 and 2013).
9) **The U-SENS™ assay**, formerly known as **MUSST (Myeloid U937 Skin Sensitization Test)**, is an *in vitro* method to assess skin sensitisation. Dendritic cell activation following exposure to sensitisers was modelled in the U937 human myeloid cell line by measuring the induction of the expression of CD86 by flow cytometry. The predictive performance of U-SENS™ was assessed via a comprehensive comparison analysis with the available human and LLNA data of 175 substances. Besides high accuracy, also an interlaboratory study showed that the U-SENS™ assay may be a promising tool in a skin sensitisation risk assessment testing strategy (Piroird *et al.*, 2015).
10) **VITOSENS** is an *in vitro* assay that models the immune recognition of chemical allergens in dendritic cells. It has been developed based on the differential expression of cyclic adenosine monophosphate-responsive element modulator and monocyte chemotactic protein-1 receptor transcripts in CD34 progenitor-derived dendritic cells, which allows classifying chemicals as skin (non-)sensitising. However, skin sensitisation is not an all-or-none phenomenon, and up to now, the assessment of relative potency can only be derived using the *in vivo* LLNA (Hooyberghs *et al.*, 2008).

Quantitative risk assessment (QRA)[1] method is under development. The basic principles of the QRA have been discussed in the SCCP Opinion SCCP/1153/08. In brief, after

[1]In essence, the dose of a sensitising chemical that is not expected to cause induction of sensitisation is adjusted by a number of safety factors in order to calculate an acceptable exposure level (AEL). In addition, a consumer exposure level (CEL) is calculated. Then the AEL is compared with the CEL, whereby, for an acceptable risk, the AEL should be greater than or equal to the CEL. To arrive at the AEL, information about the sensitising potential is obtained from the different sources discussed above. A No Expected Sensitising Induction Level (NESIL) may be

refinement and validation, the QRA approach may in the future be applicable for risk assessment of new substances (see also SCCS/1459/11). In such cases an independent post-marketing surveillance system would be essential.

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.

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 studies (EC B.30, OECD 452)
   Combined chronic toxicity/carcinogenicity studies (EC B.33, OECD 453)

In the case of the development of cosmetic ingredients which will be in contact with human skin and *mucosae* repeatedly, the SCCS is convinced that evaluation of the systemic toxicity is a key element in safety assessment.

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. Studies for a duration of 90 days or more should be used in safety assessments of cosmetic ingredients. If studies of only 28-day duration are available, a default assessment factor of 3 to extrapolate from subacute (28 days) to subchronic (90 days) toxicity may be used in the calculation of the MoS (ECHA, 2012a).

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 may become manifest.

The inhalation route is only rarely used in repeated dose toxicity testing of cosmetic ingredients due to the lack of relevance of this route of repeated exposure for the majority of cosmetic products.
This exposure route is however important where a cosmetic product is intended to be used in an aerosolised, sprayable, or powdered form that could lead to exposure of the consumer via inhalation (see Sections 3-14 and 4-3.5).
For some cosmetic ingredients, dermal repeated dose toxicity studies are submitted. These studies are taken into consideration by the SCCS. In practice, oral route studies are often used for the MoS calculation when adequate systemic exposure is achieved.

derived from animal and human data, whereupon a number uncertainty factors (Sensitisation Assessment Factors, SAF's) are applied.

In repeated dose toxicity studies, the target(s) organ(s) and critical endpoint(s) may be identified. The critical endpoint is defined as the first (in terms of dose level) adverse effect observed with the substance. This effect should be biologically relevant for human health and also in the context of cosmetic exposure. For example, local effects on the gastrointestinal tract, sometimes observed with irritants after oral exposure, are not considered relevant by the SCCS to be used for the MoS calculation. A BMD, NOAEL or LOAEL is then derived for each study. If the dose regimen of a study was 5 days treatment per week, the derived dose-descriptor corrected by a factor of 5/7 will be used. A key study (the more relevant one in terms of duration of exposure, quality of the study, levels of the BMD/NOAEL/LOAEL...) is then selected by the SCCS to be used for the safety assessment (see Section 3-12.1).

Until now, the SCCS has rarely used the BMD approach. The SCCS recognises that the BMD approach can be used as an alternative to the NOAEL approach for deriving a Point of Departure, since it makes extended use of available dose-response data and it provides a quantification of the uncertainties in the dose-response data. However, OECD guidelines are not well adapted to value this approach (more doses and less animals per group would be more appropriate). There are still practical considerations regarding the use of this approach when evaluating ingredients and its application requires a level of expert judgement and modelling expertise.

For repeated-dose toxicity testing, currently no validated or generally accepted alternative method is available for replacing animal testing. There have been efforts in the domains of *e.g.* hepatotoxicity, neurotoxicity and nephrotoxicity, but to date, no method or screening battery has been formally pre-validated (Adler *et al.*, 2011; JRC 2014).

It is self-evident that animal use should be limited to a minimum, but from a scientific point of view, this should never be at the expense of consumer safety. The SCCS considers that in case of a new cosmetic ingredient for which no repeated-dose toxicity data or a weight of evidence approach exist, the use of animal experiments to study potential toxic effects remains a scientific necessity.

For the conduct and use of animal *in vivo* studies for safety assessment of cosmetic ingredients see Section 3-1 and the scheme in **Appendix 3**.

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.

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 is also 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).

Recently, the Extended One-Generation Reproductive Toxicity Study (EOGRTS) has been adopted by the OECD (OECD 443) and a Guidance Document has been established (OECD 2013). The EOGRTS has been developed because it offers several advantages compared to older OECD TGs addressing fertility and reproductive toxicity:

- Compared to OECD TG 416 a significant number of animals can be saved.

- Many more parameters compared to older OECD TGs addressing fertility and reproductive toxicity are addressed (*e.g.* clinical-chemical parameters as normally addressed in repeat-dose studies; developmental immunotoxicity and developmental neurotoxicity in case such cohorts are included)
- It includes some new endpoints sensitive to endocrine disruption which are not included in the updated version of the two-generation reproduction study, such as nipple retention, ano-genital distance at birth, vaginal patency and balano-preputial separation
- Increased statistical power with respect to parameters for reproductive toxicity
- Possibility for modification *e.g.* to include new endpoints for the assessment of endocrine active chemicals disrupting the hypothalamus-pituitary-gonad (HPG) axis, the somatotropic axis, the retinoid signalling pathway, the hypothalamus:pituitary:thyroid (HPT) axis, the vitamin D signalling pathway and the peroxisome proliferator-activated receptor (PPAR) signalling pathway

A two-generation reproduction toxicity test is generally not submitted for cosmetic substances. On a case-by-case basis, it may be necessary to require 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:

- The Whole Embryo Culture test (WEC)
- The MicroMass test (MM)
- 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 an animal test and 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, they cannot be used for quantitative risk assessment (Marx-Stoelting *et al.*, 2009). The EST is used as a screening test.

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

In 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, there is still a need for much more information and research 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; JRC 2014a).

For reproductive toxicity testing, currently no validated or generally accepted alternative method is available for replacing animal testing (Adler *et al.*, 2011; JRC 2014a). For the conduct and use of animal *in vivo* studies for safety assessment of cosmetic ingredients see Section 3-1 and the scheme in **Appendix 3**.

It is self-evident that animal use should be limited to a minimum, but from a scientific point of view, this should never be at the expense of consumer safety. The SCCS considers that in case of a new cosmetic ingredient for which no reproductive toxicity data or a weight of

[1] http://www.reprotect.eu/, consulted September 2015

evidence approach exist, the use of animal experiments to study potential toxic effects remains a scientific necessity.

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 rearrangement 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 do not have an exact multiple of the haploid number (2006/1907/EC).

Genotoxicity is a broader term and refers to processes which alter the structure, information content or segregation of DNA and are not necessarily associated with mutagenicity. Thus, tests for genotoxicity include tests which provide an indication of induced damage to DNA (but not direct evidence of mutation) via, for example sister chromatid exchange, DNA strand breaks, DNA adduct formation or mitotic recombination, as well as tests for mutagenicity (see also 2006/1907/EC, ECHA 2015).

Based on recommendations of international groups of scientific experts (Dearfield *et al.*, 2011), and in consensus with another European Scientific Committee (EFSA, 2011) and the UK Committee on Mutagenicity (COM, 2011), the evaluation of the potential for mutagenicity of a cosmetic substance to be annexed in the Regulation (EC) No 1223/2009 should include tests to provide information on the three genotoxic endpoints, namely 1) mutagenicity at the gene level, 2) chromosome breakage and/or rearrangements (clastogenicity), and 3) numerical chromosome aberrations (aneuploidy). For this task only genotoxicity tests, which measure an irreversible mutation endpoint (gene or chromosome mutations), should be used. Indicator tests, which measure DNA damage without taking into account the consequences of this primary damage, can only provide confirmative evidence and should not be used as stand-alone tests. Finally, before undertaking any testing, a thorough review should be carried out of all available data on the substance under assessment.

Evaluation of several databases demonstrated that an increase in the number of tests performed results in an increase of the number of 'unexpected positives' while the number of 'unexpected negatives' decreases (Kirkland *et al.*, 2005). The sensitivities of the 2- and 3-test batteries seem quite comparable (Kirkland *et al.*, 2011). Moreover, the combination of the bacterial reverse mutation test and the *in vitro* micronucleus test allowed the detection of all relevant genotoxic carcinogens and *in vivo* genotoxicants for which data existed in the databases used (Kirkland *et al.*, 2011). Consequently, EFSA recommended the use of these 2 tests as a first step in genotoxicity testing for food and feed safety assessment (EFSA, 2011) and the UK Committee on Mutagenicity for stage 1 *in vitro* testing (COM, 2011). With regard to further *in vivo* mutagenicity/genotoxicity testing see the provisions on the animal testing ban for cosmetic ingredients in Section 3-1 and **Appendix 3**.

In line, the SCCS recommends two tests for the base level testing of cosmetic substances, represented by the following test systems:

- Bacterial Reverse Mutation Test (OECD 471) as a test covering gene mutations
- *In vitro* Micronucleus Test (OECD 487) as a test for both structural (clastogenicity) and numerical (aneugenicity) chromosome aberrations

Tests should be performed according to the OECD test guidelines.

In cases where the bacterial reverse mutation test is not suited (*e.g.* nanoparticles, biocidal compounds and antibiotics), a scientific justification should be given and a gene mutation test in mammalian cells (*hprt* test, mouse lymphoma assay) should be performed.

If the results from both tests are clearly negative in adequately performed tests, it is very likely that the substance has no mutagenic potential. Likewise if the results from both tests are clearly positive, it is very likely that the substance has mutagenic potential. In both cases further testing is not necessary. If one of both tests is positive, the substance is considered an *in vitro* mutagen. Further testing can be used to better assess the mutagenic (and/or clastogenic) potential of the substance under investigation.

Recently it was reported that, in the case of a positive bacterial reverse mutation test accompanied with negative results in both the mammalian *in vitro* micronucleus test and the mammalian cell gene mutation test, thus covering the genotoxic endpoints, gene mutations, structural chromosome aberrations and aneuploidy, it is unlikely that this Ames-positive substance is an *in vivo* genotoxin and/or a genotoxic carcinogen (Kirkland *et al.*; 2014). Likewise, if both mammalian cell tests are positive, it is likely that the substance possesses *in vivo* genotoxic or carcinogenic potential. Obviously, a gene mutation test in mammalian cells is a justified and valuable additional further test.
Other alternative tests in the case of a positive gene mutation test in bacteria are the comet assay in mammalian cells or on 3D-reconstructed human skin. To evaluate a positive result in the *in vitro* micronucleus test, the performance of the micronucleus test on 3D-reconstructed human skin or the comet assay in mammalian cells or on 3D-reconstructed human skin could be considered. In all these cases it is not self-evident that negative results from these alternative tests on their own overrule the positive results from a recommended test. Expert judgement may be mandatory to come to a conclusion. Mechanistic investigations (toxicodynamics and toxicogenomics) or internal exposure (toxicokinetics) may be helpful in a weight of evidence evaluation.
Alternative tests for which no OECD test guideline is available should be performed according to the general principles laid down in OECD test guidelines.

In cases where a clear positive result cannot be overruled in a weight of evidence approach even with additional testing, the substance has to be considered a mutagen. A positive *in vitro* result in genotoxicity testing is seen as indicative for the carcinogenic potential of substances.

The SCCS has published an Addendum to the SCCS's Notes of Guidance (NoG) for the Testing of Cosmetic Ingredients and their Safety Evaluation, 8th Revision (SCCS/1501/12), in which details such as definitions, critical steps, crucial experimental conditions to be followed, etc. are described (SCCS/1532/14).

3-4.8 Carcinogenicity

Substances are defined as carcinogenic if they, after inhalation, ingestion, dermal application or injection, induce tumours (benign or malignant) or increase their incidence, malignancy or shorten the time before tumour occurrence (ECB, 2003). Carcinogens are often differentiated between "genotoxic carcinogens" for which the most plausible mode of carcinogenic action includes the consequences of genotoxic effects (ECB, 2003) and "non-genotoxic carcinogens" which are carcinogenic due to mechanisms other than direct interactions with DNA.

Under the testing/marketing ban taken up in the EU Cosmetic Regulation, *in vivo* testing is prohibited for the purpose of this Regulation. The decision on the carcinogenic potential of mutagenic or genotoxic substances may be made on the outcome of *in vitro* mutagenicity tests. A positive *in vitro* result in mutagenicity testing is seen as indicative for the carcinogenic potential of substances.

At present generally accepted alternative *in vitro* methods with OECD test guidelines to determine the carcinogenic potential of substances are not available. However, there are promising new *in vitro* approaches which may be helpful to recognise genotoxic as well as non-genotoxic carcinogenic substances.
The Cell Transformation Assay (CTA) measures cell transformation that is one step in the multistep cancer process. It may provide additional information and may be used as a follow-up assay for confirmation of *in vitro* positive results from genotoxicity assays, typically as part of a weight of evidence assessment (Doktorova *et al.*, 2012). Two Guidance Documents on cell transformation assays have been drafted at the OECD to allow the scientific and regulatory communities to use the described method as part of a weight of evidence approach in the testing of substances for carcinogenic potential. These are the "*In vitro* Syrian hamster embryo cell transformation assay", which has recently been adopted (OECD 2015) and the "*In vitro* Bhas 42 cell transformation assay" (the Bhas 42 cell line was established by the transfection of the v-Ha-ras oncogene into the BALB/c 3T3 A31-1-1 cell line). The carcinogenic potential of a substance cannot be derived from a stand-alone CTA.

Without the 2-year bioassay (OECD 451), it is very difficult if not impossible to conclude on the carcinogenicity of substances. As far as genotoxic substances 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-screening for carcinogenicity. A positive result in one of the genotoxicity tests may be indicative for considering a substance as putatively carcinogenic. In combination with the CTA, this indication may be stronger.

The situation is different for the non-genotoxic carcinogens. Before the animal testing and marketing ban, they may have been detected by carcinogenicity or by chronic repeated dose toxicity studies. Alternatives for these *in vivo* tests to detect non-genotoxic carcinogens, however, are not available with the exception of the CTA but discussions are still ongoing with respect to its use as a test for non-genotoxic carcinogens.

Worldwide research is ongoing with regard to *in vitro* toxicogenomics for the detection of mutagens, genotoxic carcinogens, and particularly non-genotoxic carcinogens. The idea is that by global gene expression profiling via microarray technology, gene patterns covering diverse mechanisms of substance-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.*, 2012; Magkoufopoulou *et al.*, 2012). In addition to *in vitro* mutagenicity/genotoxicity tests (see above), data from *in vitro* tests combined with toxicogenomics may also be considered in a weight of evidence approach.

3-4.9 Photo-induced toxicity

1) Photo-toxicity (photo-irritation) and photo-sensitisation

The "3T3 Neutral Red Uptake Photo-toxicity 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.
The method has been formally validated and taken up in Regulation EC 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 Photo-toxicity 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 photo-toxicity 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 photo-clastogenicity/ photo-mutagenicity, photo-allergy or photo-carcinogenicity.

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

A post-validation exercise of the 3T3 NRU PT took place since false positives were observed, in particular for pharmaceutical substances. Some measures (*e.g.* limit of 100µg/ml as highest concentration) were taken to decrease this number (Ceridono *et al.*, 2012).

Presently, no validated *in vitro* methods for detection of photo-sensitisation are available. Nevertheless, it is expected that chemicals showing photo-allergic properties are likely to give positive reactions in the 3T3 NRU PT test (EC B.41).

Animal tests:
At present, with the prohibition of animal testing for cosmetic purposes in Europe, no official guideline-based protocols for phototoxicity testing in animals have been evaluated. Several industry reports describe test protocols. For pharmaceuticals, guidance on such testing is available (FDA, 2015; EMA, 2012). These documents do, however, not specify protocols for the testing of adverse effects of orally or topically applied agents, nor do these give recommendations about the species to be used.
Photo patch-testing of chemicals and/or cosmetic ingredients on animal skin has been described in various publications (Forbes, 1977; Lovell, 1992; Nilsson, 1993). Animals that have been used are, in decreasing order of sensitivity, hairless mice, guinea pigs, rabbits, swine. For dose-finding studies the extrapolation of the test results to humans can be problematic, although hairless mice and guinea pigs seem to be more sensitive than humans.

2) Photo-mutagenicity / Photo-clastogenicity

In 1990 the SCCS adopted guidelines for testing the photo-mutagenicity/photo-genotoxicity of UV radiation absorbing cosmetic substances.

The SCCNFP has recommended that the test protocols used by Colipa should 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 photo-mutagenicity/photo-genotoxicity, 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 photo-mutagenicity, but no results are yet available.

A 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 Averbeck *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 photo-mutagenicity/photo-genotoxicity was summarised in a review of Brendler- Schwaab *et al.* (2004), 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 photo-mutagenicity/photo-genotoxicity tests as being valid (Brendler-Schwaab, 2004).

Taking the GUM Task Force results into consideration, the SCCS evaluates the individual photo-mutagenicity/photo-genotoxicity 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 Section 3-4.7.

With respect to the evaluation of photoxicity from pharmaceuticals, the FDA and the EMA have stated that photogenotoxicity is not recommended as part of the standard photosafety testing programme (EMA, 2012; FDA, 2015). According to the FDA, experience with photoclastogenicity tests since the CPMP/SWP guideline was issued has indicated that these tests are substantially oversensitive and even incidences of pseudo-photoclastogenicity have been reported.
Considering the above and also referring to a discussion paper by EMA (EMEA, 2009), it is clear that the validity of photo-genotoxicity testing is increasingly being questioned.

3-4.10 Human data

Cosmetic products used by the consumer are substances or mixtures of substances intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, etc.) or with the teeth and the mucous membranes of the oral cavity. Occasionally, undesirable 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 frequently observed reactions.
It is inconceivable that toxicity tests in human volunteers would replace animal tests. Tests in animals and alternative methods may be 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.

With respect to bioavailability and systemic toxic effects of a cosmetic ingredient, human data might also be obtained from various sources of information: postmarketing surveillance data, results from biomonitoring programs (see also Section 3-4.11), case reports, occupational surveillance data and occupational disease registries (*e.g.* from production of the ingredient or when the cosmetic ingredient is also used in non-cosmetic areas), poison centre information, epidemiological studies, clinical studies etc.

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 sensitisation during such testing. Due to the uncertainties mentioned above, it is the opinion of the SCCS that predictive human sensitisation tests should not be carried out.

The same ethical restrictions apply to human predictive tests on photosensitisation. Information on photosensitisation (i.e. a contact allergic response to a substance that has become a sensitiser upon activation by UV light) can be obtained from published clinical studies and case reports.

In practice, phototoxicity (photo-irritation) is much more common compared to photosensitisation. Because of the absence of a lasting immunological response, testing on humans is less restrictive. It should be noted that the term 'photosensitivity' or 'photosensitiser' is in many documents used to encompass the true photosensitisation as well as the photoxicity.

There are no officially adopted guidelines or protocols, but in general the test procedures are quite similar to those that are used in photo-patch testing in clinical settings (Bruynzeel, 2004). Normally a UV-A dose of 5 – 10 J (and occasionally UV-B in appropriate non-erythemogenic dose) is applied to a skin area that has been exposed to the product or substance during the preceding 24 hours. Adequate control test areas, including a vehicle exposed and an unexposed UV irradiated area, are essential. Readings must be performed at least at 4, 24 and 48 hours after irradiation.

3-4.11 Human Biomonitoring

3-4.11.1 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).

3-4.11.2 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 minimise 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.

For cosmetic ingredients, the risk of systemic side effects is largely determined by the absorption of cosmetic ingredient across the skin as estimated by *in vitro* dermal/ percutaneous absorption studies. In case of uncharged small-size lipophilic substances, there may be a significant absorption, which may be a cause of concern for low-dose biologically active molecules. In that situation, studies measuring the unchanged compound or its metabolite in urine or blood of volunteers may be valuable. These studies may provide an accurate estimate of the systemic effective dose in humans under in-use conditions by

integrating exposure from all routes. They may also provide insight into the biotransformation and elimination rate of the substance, i.e. toxicokinetic aspects that with the ban of animal studies will be increasingly difficult to document.

For aggregated exposure, biomonitoring data may be useful to estimate internal dose of exposure resulting from different sources and route of exposure (oral, skin contact, inhalation…). Quantification of exposure by biomarkers is increasingly used to provide an integrated measure of a person's multiple chemical-specific exposures. A biomarker of exposure should be chosen to best represent usual personal exposures. Pharmacokinetics should also be taken into account. For example, non-persistent, semi-volatile chemicals are metabolised quickly. Urine is the compartment with the highest concentration of metabolites.

Progress, especially in the analytical field, has led 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 specimen 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 should rather 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 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 2014), Biological Exposure Indices or Biological Equivalents (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. Especially in view of the prohibition of *in vivo* animal studies on cosmetic substances, HBM makes it possible 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 is available, intraspecies variation can also be addressed using HBM.

3-4.11.3 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).

3-4.11.4 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 assessment 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, which is particularly important for safety evaluation as animal experiments are banned. Ethical aspects of HBM have to be handled according to national/international rules.

For toxicokinetic studies in human volunteers see the introductory part of Section 3-4.1 and Section 3-4.1.2.

3-5 TOXICOLOGICAL DATA REQUIRED FOR INCLUSION OF A SUBSTANCE IN ONE OF THE ANNEXES TO REGULATION (EC) NO 1223/2009

3-5.1 General requirements

When a dossier of a cosmetic ingredient is submitted for evaluation, the SCCS should be provided with the information set out below (the order is as given in **Appendix 2**):

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(if available);;*
8. *Reproductive toxicity(if available);;*
9. *Toxicokinetics (if available);*
10. *Photo-induced toxicity;*
11. *Human data (if available).*

Photo-induced toxicity data (point 10.) are required when the cosmetic ingredient in a cosmetic product is expected or intended to being used on sunlight-exposed skin. Human data (point 11., clinical and epidemiological studies, post marketing surveillance data and case reports, exposure data and toxicokinetic studies, etc.) may be useful or even necessary case by case.
With regard to the animal testing ban for cosmetic ingredients, see Section 3-1 and the scheme in **Appendix 3**. For alternative methods, see Section 3-4.

A retrospective study of the Annex (to Cosmetic Regulation) substances present in the opinions (2000-2014) of the SCCS and its predecessors, has shown that the cosmetic ingredients characterised by the following physicochemical properties:

- MW>500 Da,
- High degree of ionisation,
- Log P_{ow} ≤-1 or ≥4,
- Topological polar surface area > 120 $Å^2$,
- Melting point > 200°C

may be indicative of low or very low dermal absorption. In addition to these physicochemical properties, data on low oral absorption may also be used as an indicator of low or very low dermal absorption.

In the case where a cosmetic ingredient has such properties, it seems reasonable that some studies can be waived since systemic exposure via dermal absorption is expected to be minimal.
In such a case, the following minimum set of data should be made available in order to assess the safety of cosmetic ingredients with very low bioavailability:

- Experimentally determined physicochemical data
- *In vitro* dermal absorption studies, according to the SCCS Basic Criteria
- Local toxicity
- Mutagenicity/Genotoxicity.

This is a pragmatic approach which is applied on a case-by-case basis, provided that there is sufficient evidence of very low dermal or oral bioavailability of the cosmetic ingredient under consideration.

Data should be obtained by means of studies conducted in accordance with test guidelines reported in Regulation (EC) No 440/2008 (2008/440/EC) and amending ATP Regulations, as well as the OECD test guidelines, and complying with the principles of Good Laboratory Practice. All **possible deviations** from validated methods or from GLP must be indicated, explained and scientifically justified.

There may be cases for which it is either not necessary or technically not possible to provide some of the information mentioned above: in such cases **a scientific justification** must be given.

It should be further noted that:

- Whenever study results are submitted, a declaration should be made that the tests involved were conducted using a cosmetic ingredient with a comparable purity/impurity profile and physical and chemical characteristics of that to be included in the finished cosmetic product.
- Stability of the test substance under experimental conditions is of prime importance for the interpretation of test results.
- The stability of the test material under conditions of use should also be reported.
- Ensuring that files for evaluation are complete when submitted is an important requirement. The applicant should 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 (cosmetovigilance data);
  - all relevant published literature;

- a description of the bibliographical methods used;
- any useful finding to the applicant's best ability;
- any information from "grey material" available elsewhere.

- Any new information acquired by industry and/or relevant agencies, should be transmitted to the Commission for review.
- In their dossiers, applicants should indicate data/tables that they consider confidential (typically impurities etc.) for commercial reasons and provide relevant codes to be used by the SCCS members as they may comment on the confidential data indicated.

In the following sections, some general issues, caused by the nature and/or origin of the cosmetic substances under consideration, are discussed.

3-5.2 Specific Requirements for Safety Assessment of Ingredients of Natural Origin

Many cosmetic ingredients are chemical mixtures. For instance, essential oils and fragrances are often chemical mixtures of natural origin, which may considerably vary in their composition depending on their geographical origin, conditions of harvest, storage, further technical processing etc. In such cases, the cosmetic ingredient should contain the following information:

- semi-quantitative concentrations of the substances in the mixture (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

  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; or

  3) a clear indication of the types of cosmetic products in which the compound may be used and at what maximum concentration.

In the final risk evaluation, reference should be made to the semi-quantitative composition of the cosmetic ingredient/chemical mixture 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 makes it possible to derive a general threshold for substances with a higher number of recorded cases (SCCS/1459/11).

3-6 POTENTIAL ENDOCRINE DISRUPTORS

Definitions and background

Substances with the ability to interact or interfere with one or more components of the endocrine system may exert harmful effects on human or animal health (Damstra *et al.*; 2002; UNEP WHO, 2013). The so-called "endocrine disruptors"(EDs) have been subject to intensive scientific investigation and discussion, and several working **definitions** have been suggested. The SCCS, in accordance with other scientific bodies of the European Commission (EFSA, 2013; JRC, 2013b), endorses the definitions of WHO/IPCS:

> *"An **endocrine disruptor** 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".*

and

> *"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."*

These definitions are important both in the context of testing for endocrine activity and evaluating substances for endocrine disruption, and have been taken up in two recent reports on scientific issues relevant for the hazard assessment (identification and characterisation) of endocrine active/disrupting substances (EFSA, 2013; JRC, 2013b,c): the proposed elements for the identification of endocrine disrupting substances should be the demonstration of an adverse effect for which there is convincing evidence of a biologically plausible causal link to an endocrine disrupting mode of action. Therefore, no single model or assay is likely to provide all the information needed to decide whether a substance is an ED, since information on both the mechanisms/mode of action and the adverse effect will be required (EFSA, 2013b; JRC, 2014a).

The OECD has developed a guidance document and (enhanced) testing guidelines for evaluating chemicals for endocrine disruption (OECD, 455; OECD, 457). An overview on the OECD Conceptual Framework for tiered testing (at 5 levels) is provided in **Appendix 4** of the Notes of Guidance. Further details are provided in recent reviews on the identification/characterisation of EDs by EFSA (EFSA, 2013; Annex C.2) and by JRC (Section 11 in JRC, 2014a).

Evidence for an ant-/agonist activity in *in vitro* screening assays would mark a substance as **potential ED** which may be confirmed by *in vivo* assays that provide data on selected mechanisms and hormonal potency (*e.g.* Uterotrophic and Hershberger assays). But, to obtain data on adverse effects on endocrine relevant (apical) endpoints, repeated dose toxicity studies, including those which cover susceptible exposure periods, are indispensable.

Cosmetic ingredients suspected to have endocrine disrupting properties

There is not yet a harmonised approach on health risk assessment procedures for endocrine active substances within the different regulatory frameworks in the EU[1]. Whilst there is general consensus on the WHO/IPCS definition of an ED, and although provisions on ED are in force in some sectorial EU legislation (*e.g.* Biocides Regulation), no formal criteria have been established for identifying an ED.

Recently, the SCCS has issued a memorandum (SCCS/1544/14) to clarify its position on substances with endocrine disrupting properties when used as cosmetic ingredients: as emphasised there, they should be treated like most other substances of concern for human health and be subject to risk assessment and not only hazard assessment.

[1] http://ec.europa.eu/smart-regulation/impact/planned_ia/docs/2014_env_009_endocrine_disruptors_en.pdf (Roadmap – published 06/2014 by DG ENV.A.3 and DG SANCO.E.3 Defining criteria for identifying Endocrine Disruptors in the context of the implementation of the Plant Protection Product Regulation and Biocidal Products Regulation

This position is in agreement with past and present practices of the SCCS with regard to the safety assessment for substances with suspected endocrine disrupting properties. Examples of cosmetic ingredients evaluated by the SCCS and its predecessors (SCCP and SCCNFP) are several parabens (SCCP/1017/06, SCCP/1183/08, SCCS/1348/10, SCCS/1446/11, SCCS/1514/13), triclosan (SCCP/1192/08, SCCS/1414/11), homosalate (SCCP/1086/07), benzophenones, 4-methylbenzylidene camphor and 3-benzylidene camphor (SCCNFP/0483/01, SCCP/1183/08, SCCS/1513/13), melatonin (SCCS/1315/10), resorcinol (SCCS/1270/09) and cyclomethicone (SCCS/1241/10), i.e. substances used as preservatives, UV-filters and for other functions. The opinions illustrate the types of data needed in a scientific evaluation of substances suspected to have endocrine disrupting properties: data from *in vitro* studies suitable to detect different hormonal activities were reviewed together with data from *in vivo* studies relevant for detection of related developmental and reproductive toxicity as well as information on human exposure resulting from the use of these substances. Thereby, conclusions are made whether endocrine/hormonal activities are linked to the critical endpoint for assessing the safety of these substances for consumers, including vulnerable groups such as children when applicable.

Due to the ban on animal testing for cosmetic ingredients effective since 2013, it will be extremely difficult in the future to differentiate between a **potential ED** and **ED**, if the substance is only used in cosmetic products (see Section 3-1, **Appendix 3** and **Appendix 4**). 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; JRC, 2014a).

With regard to substances with endocrine activity (potential endocrine disruptors), the assessment of their impact on human health without animal data remains a challenge. A way forward may be demonstration of what could be considered as biologically irrelevant exposure. For instance, in the case of melatonin, topical application (in real use conditions) did not perturb endogenous hormone levels in humans due to low systemic exposure (SCCS/1315/10). Toxicokinetic studies and PBPK modelling could help to bridge the gap between *in vivo* and *in vitro* by providing data on (internal) exposure in relation to concentrations which were found to be active in *in vitro* assays (Coecke *et al.*, 2013; Bessems *et al.*, 2014).

3-7 CMR-SUBSTANCES

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 1272/2008 (2008/1272/EC).

CMR 1A, 1B and 2 substances are prohibited for use in cosmetics, unless the specific criteria set in Cosmetics Regulation (EC) No 1223/2009 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 [1], (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.

[1] 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

A Guidance document has been developed by the EU Commission with the aim of enabling a harmonised approach to the development and use of aggregate exposure estimates in assessing the safe use of CMR substances as cosmetic ingredients (see **Appendix 5**). However, as a clarification and agreed by the Commission, whereas the applicant is responsible for providing the exposure data on CMR substances, the procedure described in No. 16-19, 21 and 22 of the Guidance, is **only** foreseen in case that the applicant for any reason cannot obtain the data from the owner of the data required.

3-8 NANOMATERIALS

The use of nanomaterials in cosmetics is subject to a high level of protection of human health under the Cosmetics Regulation (EC) No 1223/2009. This is because nano forms of some substances may differ from their conventional forms in terms of physicochemical properties, biokinetic behaviour, and/or biological effects. Whilst a brief guidance is provided on this subject in this section, the SCCS has published a more detailed Guidance on Risk Assessment of Nanomaterials (SCCS/1484/12), and a Memorandum on the Relevance, Adequacy and Quality of the Data Expected in Safety Dossiers on Nanomaterials (SCCS/1524/13, Revision of 27 March 2014). Safety assessors need to consult these documents to ensure that any testing to generate evidence on safety of nanomaterials is carried out with special considerations to the nano-size related characteristics of the materials.
It is also important to note that the SCCS will only consider the data provided in a dossier which are relevant to the nanomaterials under evaluation, sufficiently complete, and of appropriate quality to facilitate risk assessment.

The SCCS has also recently published scientific opinions on nano-form of 1,3,5-Triazine, 2,4,6-tris[1,1'-biphenyl]-4-yl- (ETH50)[1]; zinc oxide[2]; titanium dioxide[3]; and carbon black[4]. These opinions can provide further information on the type of scientific evidence needed in a safety dossier on nanomaterials.

In brief, the EU Cosmetics Regulation (Regulation (EC) No 1223/2009) specifically covers the use of nanomaterials in cosmetic products. The Regulation provides a definition of nanomaterials, as well as a mechanism for notification, labelling, and safety evaluation of cosmetic products containing nanomaterials. In 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 either completely soluble or degradable and hence not persistent in biological systems (*e.g.* liposomes, oil/water 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[5]. 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

[1] http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_070.pdf
[2] http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_103.pdf
[3] http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_136.pdf
[4] http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_144.pdf
[5] https://ec.europa.eu/research/industrial_technologies/pdf/policy/commission-recommendation-on-the-definition-of-nanomater-18102011_en.pdf

number size distribution, one or more external dimensions is in the size range 1 nm – 100 nm [1]*.*

Detailed and technical information about the definition of a nanomaterial is available in the "questions and answers" section[2]. 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, the Scientific Committee on Emerging and Newly Identified Risks (SCENIHR) adopted an opinion on the appropriateness of the current methodologies 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 nanomaterials. However, it has also been pointed out that the current testing methods may need certain adaptations to take account of the special features of nanomaterials (Rocks *et al.*, 2008; SCENIHR, 2009; OECD, 2009c). This is because:

- Due to high surface energies, nanoparticles tend to stick together to form larger agglomerates and aggregates, or bind with other moieties. However, this particle behaviour can also change in the presence of certain stabilising/dispersing agents. Therefore, composition of a test medium may lead to substantial changes in the degree of aggregation/ agglomeration of nanoparticles during the test, and may thus affect the results. Characterisation of nanomaterials, prior to and during a test, is therefore a key to ensuring that valid results are obtained.
- Most test methods have been developed and are suitable for substances that are soluble. In contrast, insoluble and poorly-soluble nanomaterials are present in a test medium as a nano-suspension rather than a solution. The applied concentration of a nanoparticle may therefore drop during a test due to agglomeration, sedimentation, binding with other moieties in the medium, or sticking to the sides of the glass/plastic ware. This requires ascertaining the stability of a nano-suspension so that the applied concentration of a nanomaterial is maintained during the test to ensure uniform exposure of the biological system.
- Nanomaterials are also known to adsorb or bind different substances on their surfaces, including proteins (Šimon and Joner 2008; Lynch and Dawson 2008). They may also bind other substances in a test medium and carry them into the exposed test systems, leading to artefacts. Again, adequate characterisation of nanomaterials, and the use of appropriate controls, should be ensured so that a test does not generate erroneous or questionable results.
- The toxicological hazards of chemical substances are measured and expressed in weight or volume units (such as mg/kg, or mg/l). These conventional metrics may not be appropriate for nanomaterials. Discussions around identification of appropriate dose metrics for nanomaterials are currently ongoing. Until suitable parameters are identified, it is important that tests on nanomaterials are evaluated using different dose-describing metrics, such as weight/volume concentration, particle number concentration, surface area etc.
- Due to the insoluble particulate nature and the nano-dimensions, nanomaterials may have an altered uptake and biokinetic profile in a biological system compared to equivalent conventional forms. The potential ability of nanoparticles (especially in the lower nm range) to penetrate cellular membrane barriers has added a new dimension to particle toxicology. Currently, there are a number of uncertainties whether the endpoints identified by the current testing methods will be sufficient to

[1] 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.

[2] http://ec.europa.eu/environment/chemicals/nanotech/faq/questions_answers_en.htm

identify and characterise all the hazards that may be associated with a nanomaterial. For hazard identification, emphasis should therefore be on toxicological tests over prolonged periods with repeated doses that are followed up by detailed histopathological investigations. With regard to the animal testing ban for cosmetic ingredients, see Section 3-1 and the scheme in **Appendix 3.**

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 detailed Guidance on this subject has been published. This Guidance (SCCS/1484/12) is meant to facilitate the preparation of safety dossiers, and to assist the implementation of the provisions of Article 16 of the EU Cosmetic Regulation 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 in SCCS/1484/12 relates to material identification, specification, quantity, toxicological profile, exposure estimates, and safety evaluation, which needs to be provided for nano forms of any ingredients intended for use in a cosmetic product.

An exception applies for nanomaterials used as colourants, UV-filters or preservatives regulated under Article 14, as their inclusion in the Annexes is in any case subject to safety assessment by SCCS. The notification of cosmetic products containing nanomaterial has become 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 emphasised in the Guidance Document (SCCS/1484/12):

For any new or already approved cosmetic ingredient fulfilling the criteria for a nanomaterial as provided in the Cosmetic Regulation, Article 2 (1) (k), as amended, safety data will be required from tests carried out with special considerations to the nano-scale properties for risk assessment.
Irrespective of the presence of nanomaterials, the requirements under existing regulations and the SCCS Notes of 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 include measurement of important physicochemical 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 of the nanomaterial needs to be carried out at the raw material stage, in the cosmetic formulation, and during exposure for toxicological evaluations. Where needed, the SCCS may ask further information, such as the description of production processes, surface modifications/ coatings, and/or any preparatory steps carried out for integrating the nanomaterials to the final cosmetic products to facilitate risk assessment.
Although most analytical methods used routinely for chemical substances have not yet been validated for nanomaterials, a careful choice of mainstream method(s) should provide sufficient means to gather adequate characterisation data. The use of more than one method generally adds more confidence to the measured values. For this reason, the use of more than one method for measurement of particle size distribution, and particle imaging (*e.g.* by transmission electron microscopy) has been recommended by both SCCS (SCCS/1484/12) and EFSA (2011).

For *in vitro* genotoxicity assessment, both chromosomal damage and gene mutations should be evaluated. The Ames test is not considered appropriate for nanomaterial mutagenicity assessment, due to limited uptake of the nanomaterial by the bacteria (SCCS/1484/12). The bacterial cell wall hinders uptake and thus nanoparticle internalisation is unlikely to

occur to the same extent as observed in mammalian cells, hence sensitivity of the assay is questioned. It is therefore suggested that the following *in vitro* genotoxicity tests be conducted:

- Mammalian cell chromosome aberrations/clastogenicity – determined either by *in vitro* chromosome aberration test or micronucleus test. The micronucleus test can be performed using either the mononucleate or cytokinesis blocked protocols. However, if the cytokinesis blocked micronucleus assay is to be applied then cytochalasin B addition must be post-treatment (after the nanomaterial exposure period) or a delayed-co-treatment protocol is acceptable if a sufficient nanomaterial exposure period has been allowed to enable uptake into the test system cells. Co-exposure to both cytochalasin B and the test nanomaterial for the duration of the experiment should be avoided as this is not considered acceptable.
- An *in vitro* mammalian cell gene mutation test (*e.g. hprt, tk* or *xprt* tests).
- Other indicator tests, such as the Comet assay, may be included as a further weight of evidence.

Additionally, the *in vitro* genotoxicity studies should be accompanied by an assessment of cellular and nuclear uptake to demonstrate target exposure to enable a complete evaluation of data-outputs.
New *in vitro* approaches such as cell transformation assays or toxicogenomic approaches may also be useful for identification of genotoxic as well as non-genotoxic carcinogen nanomaterials.
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 this document. However, certain assumptions and models used for estimation of dermal absorption of conventional chemical ingredients are not applicable to nanomaterials. Dermal absorption of nanomaterials therefore needs to be determined experimentally.
For sprays/sprayable cosmetic products containing nanomaterials, droplet size as well as size distribution of the dried residual aerosol particles will need to be measured. For a distinction between **propellant spray** and **pump spray** see Glossary; see also the SCCS Opinion on term "sprayable applications/products" (SCCS/1539/14).
The likelihood and extent of the 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 nanoparticle form or in solubilised/metabolised form. Where the absorption of nanoparticles cannot be ruled out 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 nanoparticle form.**

Where application of a nanomaterial-containing cosmetic product can lead to systemic exposure, data on toxicological evaluation of the nanomaterial(s) will be required. Toxicological testing for hazard identification/ dose response characterisation of nanomaterials need to be carried out in consideration of the nano-related aspects. Information on the possible local effects will also be required.

Where nanomaterials can become systemically available initial focus of testing should be on determining ADME (absorption, distribution, metabolism and excretion) parameters to investigate the biokinetic behaviour and fate of the nanomaterial and to identify the likely target organs. In view of the animal testing ban for cosmetic ingredients, see Section 3-1 and the scheme in **Appendix 3** for possible alternative testing methods.
Like other cosmetic ingredients, data on a base set of toxicological endpoints is required for nanomaterials. 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

and reproductive toxicity may also be required. Photo-induced toxicity data are specifically required when a cosmetic product is expected or intended to be used 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 forms of chemical substances, and not for nanomaterials. These tests may, however, be relevant for hazard identification of nanomaterials, and provide additional supporting evidence to the results of other studies, as well as information on the possible mechanism(s) of toxic action, provided that they are carried out with due consideration of the nano-related aspects as outlined in the SCCS Nano Guidance (SCCS/1484/12). 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 (SCCS/1484/12) more detailed considerations which should be taken into account when assessing the safety of nanomaterials in cosmetic products, can be found.

With respect to MoS calculation, the 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 any higher margins of safety to a nanomaterial than those used 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.

It should also be noted that the SCCS requires relevant, adequate and quality data in safety dossiers on nanomaterials, and will not consider those nanomaterials for which data are either not provided by the applicant, or not available in the open scientific literature. In this regard, the importance of the relevance, adequacy, and quality of the data presented in the safety dossiers on nanomaterials has been highlighted in the recently published Memorandum 'Relevance, Adequacy and Quality of Data in Safety Dossiers on Nanomaterials' (SCCS/1524/13). The Memorandum is meant to provide guidance and clarity to facilitate preparation of safety dossiers in a manner that meets the standards expected by the SCCS for their evaluations.

3-9 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.

3-10 ANIMAL-DERIVED COSMETIC SUBSTANCES, INCL. BSE-ISSUES

The most recent adaptation of previous Directives to entry no. 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 1774/2002 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).

3-11 THE SPECIFIC ASSESSMENT OF HAIR DYES AND HAIR DYE COMPONENTS

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 SCCP 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).

In 2013, the SCCS confirmed the views expressed in an earlier Memorandum (SCCP 2006), that hair dye substances which fulfil the criteria for classification as Skin Sens 1, H317 (according to CLP) may not be safe for consumers and that this is particularly so for hair dye substances categorised as extreme and strong sensitisers (SCCS/1509/13).

3-11.1 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 to change to the more commonly used **scalp surface area value of 580 cm²** in its evaluations (SCCS/1416/11).

2. Intermittent exposure and MoS calculations

It is acknowledged that the calculation of 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, 2012a).

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.

3-11.2 Assessment of oxidative 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.

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 couplers. 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 + 1 SD). 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, sensitisation risk of the reaction products was not specifically 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 an 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. With regard to the animal testing ban for cosmetic ingredients, see Section 3-1 and the scheme in **Appendix 3.**

3-12 GENERAL PRINCIPLES FOR THE CALCULATION OF THE MARGIN OF SAFETY AND LIFETIME CANCER RISK FOR A COSMETIC INGREDIENT

3-12.1 Calculation of the Margin of Safety of a cosmetic ingredient

The last step in the safety evaluation of a cosmetic ingredient is the calculation of the Margin of Safety (MoS), which is the ratio between a NOAEL and an estimate of the exposure. For cosmetic ingredients, a Systemic Exposure Dose (SED) is derived as the exposure estimate. Therefore, a systemic $NOAEL_{sys}$ is also derived and the MoS is then calculated by dividing the systemic $NOAEL_{sys}$ by the SED:

$$MoS = \frac{NOAEL_{sys}}{SED}$$

The above equation consists of three important parameters:

a) The Margin of Safety (MoS)

The MoS value is compared with a reference MoS, which is comparable to the uncertainty/assessment factor used in general to extrapolate from a group of test animals to an average human being, and subsequently from average humans to sensitive subpopulations (see Fig. 2). A default value of 100 (10x10) is generally accepted and a MoS of at least 100 therefore indicates that a cosmetic ingredient is considered safe for use.

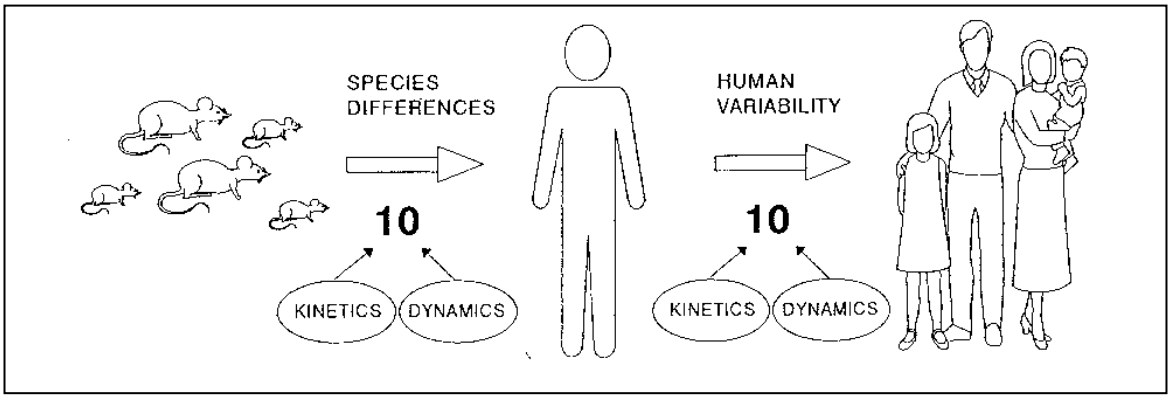

Fig. 2: Schematic representation of the extrapolation from animal to man (Renwick, 1998).

As shown in Fig. 2, the default value of 100 consists of a factor of 10 for the extrapolation from test animals to an average human being and another factor of 10 taking into account the intraspecies (interindividual) variations within the human population. These factors can be further subdivided as indicated in Fig. 3.

When considerable qualitative/quantitative toxicokinetic differences are observed between test animals and humans, as well as within human individuals, *e.g.* from relevant toxicokinetic data for rat and/or humans (SCCS/1443/11, SCCS/1479/12), the interspecies and/or intraspecies toxicokinetic default factor (see Fig. 3) can be reduced or enhanced (case-by-case evaluation) (see Section 3-4.1).

In other cases, for instance in case of different susceptibility to hypothalamic-pituitary-thyroid (HPT)-axis disturbances in rats and humans a change of the interspecies toxicodynamic default factor of 2.5 may be required (SCCS/1481/12).

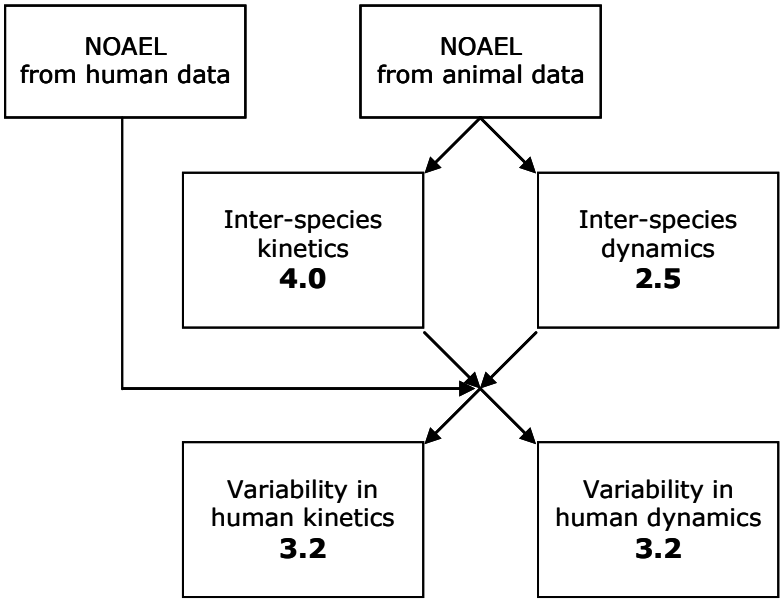

Fig. 3: Further subdivision of the uncertainty/assessment factor, taking toxicokinetics and toxicodynamics into account (based on WHO, 1994).

An additional remark with regard to MoS calculations is whether such calculations are scientifically relevant for cosmetic substances that are not used on a daily basis, i.e. cosmetics with intermittent exposure such as hair dyes. For example, comparing a usage level of *e.g.* once per week or once per month with a NOAEL value obtained after daily administration of the substance, is a clear overestimation of the risk. The comparison of a NOAEL resulting from a daily exposure study with the SED of a certain cosmetic ingredient is accepted, even if it is only applied *e.g.* once per week or 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) as justified by: "*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, 2012a).

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 judgement.

If there is sufficient evidence that the dermal absorption of a cosmetic ingredient is very low, systemic exposure may be negligible and the calculation of a MoS may not be justified or applicable (see Sections 3-4.1.1 and 3-5.1). See also for example SCCS/1533/14.

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**.**

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.

b) The Systemic Exposure Dose (SED)

Generally, the SED of a cosmetic ingredient is estimated by taking into account the amount of the finished cosmetic product applied per day, the concentration of the substance in the finished cosmetic product, the dermal absorption of that particular substance and a mean human body weight value.

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

c) The NOAEL value and other dose descriptors

The No Observed (Adverse) Effect Level (NOAEL) is defined as the highest dose or exposure level where no (adverse) treatment-related findings are observed. For cosmetic ingredients, the NOAEL is mainly derived from a 90-day repeated dose animal study or from a developmental toxicity animal study. If the dose regimen of a study was 5 days treatment per week, a NOAEL corrected by a factor of 5/7 should be used for the MoS calculation (ECHA, 2012a).
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 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, 2012a).

If a NOAEL cannot be identified from the available data, other dose descriptors such the Lowest Observed (Adverse) Effect Level (LOAEL) instead of the NOAEL may be used in the MoS calculation. Often an assessment factor of 3 is used in the calculation of the MoS for a cosmetic ingredient. However, a higher assessment factor of up to 10 may be 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 cases the extent and severity of the effect(s) seen at the LOAEL). In some cases, the study cannot be used for safety assessment.

Instead of the NOAEL / LOAEL, the BMD approach may be used as the dose descriptor for the MoS calculation (EFSA, 2009).

In case a 90-day repeated dose toxicity study is not available, a NOAEL from a 28-day repeated dose toxicity study can be used in the MoS calculation for a cosmetic ingredient. In this case, a default assessment factor of 3 for exposure duration may be used in the calculation of the MoS.

For most of the cosmetic ingredients evaluated by the SCCS, the SED is compared to an oral NOAEL. Generally, the NOAEL identified in a toxicity study corresponds to the dose that has been administered orally, i.e. the external dose. For cosmetic ingredients, the MoS is calculated by dividing the internal (systemic) $NOAEL_{sys}$ with the SED. For cosmetic ingredients it is considered that not more than **50%** of an orally administered dose is systemically available. Thus, in the absence of data, 50% of the administered dose is the default oral absorption value for a cosmetic ingredient and the $NOAEL_{sys}$ is derived from the NOAEL by dividing with a factor 2.

If there is information to suggest poor oral bioavailability, a default value of **10%** oral absorption is considered, see Section 3-12.2. Whenever oral absorption data are available, these should be used, also when using other dose descriptors.

For the safety assessment of exposures resulting from a non-oral route, route-to-route extrapolation is often done by correcting the non-oral route exposure by the route specific absorption into the systemic circulation and comparing the result with the (oral) threshold value. Making use of this procedure means that an internal dose obtained from the non-oral route (dermal or inhalation for cosmetic exposure) has to be compared with an internal dose of the oral route. If the absorption on the oral route is 100%, then the external or internal doses of the oral route are equivalent. If the absorption on the oral route is less than 100%, which is often the case, the procedure may underestimate the risk of the exposure of the non-oral route. Therefore in the case of oral to inhalation extrapolation, a default factor of 2 is proposed (default absorption oral route: 50%; inhalation 100%; ECHA, 2014b).
For chemicals with a high first pass metabolism in the gut or liver, the situation is even more complex and, in addition, the target organ for toxicity has to be taken into consideration and route-to-route extrapolation may not be adequate.

3-12.2 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 1 in Section 4-2) to estimate the systemic availability of the substance.

Calculations of the SED may also be based on the **percentage** dermally absorbed. This depends on the amount of finished product applied on the skin (see Table 2 in Section 4-2 for default values per product type). In this case, the concentrations tested should also include the lowest concentration anticipated.

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 Dose
- DA_a (µg/cm²) = Dermal Absorption reported as amount/cm², resulting from an assay under in-use mimicking conditions[1]
- SSA (cm²) = Skin Surface Area expected to be treated with the finished cosmetic product (see Table 1 in 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 Dose
- 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 2, 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.

3-12.3 MoS for children

Under certain circumstances it might also be necessary to calculate the MoS for certain subpopulations such as children (*e.g.* in case of exposure to specific cosmetic products such as leave-on cosmetic products designed for application on the nappy area or in case of indication of higher sensitivity of children for certain end-points). The question is sometimes raised whether a higher MoS (above 100) would be required in order to cover children exposed to the ingredient.

[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.

Definitions

"Children" are developing human beings who are at various stages of immaturity and maturation for up to nearly two decades, with age-dependent different susceptibilities and sensitivities (Makri *et al.*, 2004; Lemper *et al.*, 2009) compared to adults.
Terms usually covered by the word "children" include:

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

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 default assessment 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). In these calculations, the BW values determined earlier were used. In the meantime, updated values became available (EFSA, 2012).

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).

These data indicates that the difference between the SSA/BW children of 0 to 1 year of age and that of adults is at maximum a factor of 2.3. A factor of 3.2 is generally applied by the WHO covering also variability in human kinetics (see Section 3-12.1). Consequently, the inter-individual variation in SSA/BW is covered by the generally accepted default value of 100 for intact skin (Fig. 3 in Section 3-12.1). However, potential differences in metabolism between newborns/infants up to six months and adults require consideration. In general, there is no need for an additional uncertainty factor for children when **intact skin** is involved (SCCNFP/0557/02).

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

Age-related susceptibilities/sensitivities

The rationale of an additional assessment factor for the different age groups beyond the usual factor of 100 has been extensively discussed in the scientific literature (*e.g.* Renwick *et al.*, 1998 and 2000; Nielsen *et al.*, 2001; Makri *et al.*, 2004; ECHA, 2012a). A number of potential risk factors do exist in the newborn and early infant. They are extensively reviewed in Annexes 1 and 3 of SCCS/1446/11 but as dermal exposure in children is a topic of high importance for several cosmetic substances, the most important points are summarised here.

Dermal exposure of the newborn and early infant[1]

- When born at full-term, the skin possesses all skin structures of adult skin, and anatomically these structures do not undergo dramatic changes after birth. The dermal absorption in skin of newborns is similar to that observed in adult skin, when the skin is intact (see SCCS/1446/11) (Visscher *et al.*, 2009).
- Differences between newborns during their first weeks and months and adults are described below:

(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 is in general covered by the intraspecies factor of 10 (3.2 x 3.2) used in the calculation of MoS.

(ii) Toxicokinetic parameters may differ between various age groups of children and adults and can result in reduced metabolism, clearance and/or longer half-life that might either increase or decrease the potential risk of an adverse reaction in newborns, depending on the substance (Renwick *et al.*, 2000; Nielsen *et al.*, 2001).

For the CYP450s in the liver, lower activities in newborns/early infants as compared to adults have been described (Johnson 2003). This data suggests that the extent of bioactivation or metabolic detoxification in children between one and ten years will in general unlikely be higher as compared to adults. It is also known that detoxification of xenobiotic substances or metabolites by phase II enzymes may be lower in newborns and infants compared to adults due to yet incomplete development of xenobiotic metabolising enzymes (XME) in the liver (*e.g.* , UDP glucuronosyltransferase 1 (UGT1A1) and some esterases; see SCCS/1446/11). Therefore, depending on the cosmetic ingredient in question, the balance between activating and inactivating XME activities may be crucial for systemic exposure. In general however, it is assumed that a specific assessment factor for age-related differences in toxicokinetics is not required (SCCS/1446/11).

With respect to skin metabolism, it is recognised that some metabolic enzymes seem to be less 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 ingredients after dermal application than adults. For a sound risk assessment, relevant human data regarding metabolism are necessary. These data could for instance be gained by an approach combining *in vitro* data on the metabolism of the cosmetic ingredient under investigation and PBPK/PBTK modelling. For such toxicokinetic modelling of the biotransformation in humans of different age groups, relevant *in vitro* data regarding phase I and phase II biotransformation are needed both in 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 no comprehensive exposure data for newborns and early infants are available in the open literature but some information is available in the RIVM (National Institute for Public Health and the Environment, the Netherlands) ConsExpo Fact Sheet (2006).

(iv) The nappy area: the skin barrier function in the nappy area and non-nappy regions are indistinguishable at birth but show differential behaviour 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 slightly higher water content in the horny layer and a greater variation than newborns, infants and crawlers

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

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 (nappy dermatitis). This 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) and may occur in particular between 6-12 months of age.
See below for cosmetic products used in the nappy area.

(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 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 Section 4-4.1).

With respect to points (i) - (iii) above, there is generally no need for an additional assessment factor for children when intact skin is involved. However, an additional assessment factor might be relevant if substance-specific data clearly demonstrate that inter-individual variability would result in a value higher than the default value of 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 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 assessment factor needs to be included for substances used in children's cosmetics on intact skin as the intra-species default assessment factor of 10, covering the toxicokinetic (3.2) and toxicodynamic (3.2) differences between children and adults, is already included in the MoS calculation for individual substances.

- The default assessment factor of 10 is usually sufficient to protect the larger part of the population, including *e.g.* children. It is recognised that there are differences between children and adults in toxicokinetics (especially newborns) and toxicodynamics (especially at different stages of development). These differences may render children more or less susceptible to the toxic effects of a substance. A higher intraspecies assessment factor for

children may be considered case-by-case in particular exposure situations (*e.g.* nappy area).

3-12.4 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 the genetic material (non-genotoxic) is a major determinant for the selection of risk assessment methodologies. Genotoxic agents are often considered not to have a threshold for their carcinogenic effect. It is therefore assumed but not proven that a very low dose of a genotoxic agent may results in an increased cancer incidence, although the increase may be very low. Therefore, at low doses and as a default, they are assumed to induce increases in DNA damage linearly related to the administered dose. Non-genotoxic carcinogens are assumed to have a threshold for their carcinogenic effect.

The distinction of a threshold or a non-threshold mode of action of a carcinogenic agent may be difficult. If a threshold mode of action is not clear, a non-threshold mode of action is assumed and thus, the safety assessment is performed as for non-threshold carcinogens.

Non-genotoxic carcinogens

In case of non-genotoxic carcinogens, where a threshold mode of action for induction of tumours has been identified, the safety assessment is performed as for other toxicological endpoints with a threshold dose descriptor and by calculation of a MoS.
Considering the current ban on animal testing, identifying non-genotoxic carcinogens by alternative methods may not be possible. With regard to the animal testing ban for cosmetic ingredients, see Section 3-1 and the scheme in **Appendix 3**.

Genotoxic carcinogens

Both the Scientific Committees ((SCs) SCHER/SCCP/SCENIHR, 2009) and the REACH guidance documents (ECHA, 2012a and 2015) provide guidance on the safety assessment of genotoxic and carcinogenic substances. Whenever sufficient information is available, an appropriate dose descriptor, T25 or BMDL10, should be identified. The T25 (expressed as mg/kg bw/d) 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 (expressed as mg/kg bw/d) uses mathematical curve fitting techniques to calculate the lower 95% confidence level at a 10% benchmark response (EFSA, 2005, 2009). Both T25 and BMDL10 can be used as starting points 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
Two methods for calculation of LCR have been used by regulatory authorities in Europe. EFSA (2005) recommends the BMDL/Margin of Exposure approach (see below). The "T25 method" (Sanner *et al.*, 2001) is used as a simple default method for quantitative risk assessment of carcinogens in the REACH Regulation (ECHA, 2012a). The results obtained with these methods are in most cases quite similar. It should be noted that, in six cases where high quality epidemiology and animal carcinogenicity studies are available, quantitative risk characterisation based on epidemiological data and data based on animal studies using the T25 method differed by factors of less than three (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 ECHA (2012a) and Dybing *et al.* (1997).

The animal dose descriptor (T25) is converted to the human dose descriptor (HT25) based on comparative metabolic rates (Sanner *et al.*; 2001):

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

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

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

The decision on the threshold for concern with regard to the calculated lifetime cancer risk is a political issue. Some countries and international organisations have considered that an LCR in the general population of less than 10^{-5} is of little or no concern (SCCS/1486/12).

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 and the following elements are reported/discussed in detail by Sanner *et al.* (2001) and ECHA (2012a).

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 data indicates that the calculated risks are clearly under- or overestimating actual risks (i.e. the data indicates a supralinear or sublinear dose-response relationship for this part of the response curve, respectively), some qualitative or quantitative judgement 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 (SCs), 2009; ECHA, 2012a) (*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*". 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.

3.13 THE THRESHOLD OF TOXICOLOGICAL CONCERN (TTC)

3-13.1 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 intended 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. A database containing carcinogenicity data from animal studies for more than 1500 chemicals (Carcinogen Potency Database) (Gold *et al.,* 1984) and a database containing 613 chemicals based on other toxicological endpoints (Munro database) (Munro *et al.*, 1996) were available when TTC evaluations took place. 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 further research is needed in each of these areas.

3-13.2 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. A number of misclassifications of compounds when using the Cramer decision tree in its present form have been revealed.

The SCs conclude that the TTC value of Cramer Class II is not supported by the currently 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 **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 time being, **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, analysing 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.

3-13.3 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 situations of very low exposure. 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 relationships.

In relation to cosmetic ingredients, 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 ingredients, 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 the meantime in the SEURAT - 1 COSMOS project (EU framework program 7), further work has been done on the non-cancer TTC for cosmetics-related chemicals. An extensive COSMOS TTC dataset, including 560 relevant chemicals (495 cosmetics) has been established and quality controlled. The SCCS will later onwards analyse this additional data.

In addition, it should be noted that an appropriate exposure assessment is essential for all risk assessments, including application of TTC. Biologically relevant 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. For cosmetic ingredients, the TTC approach should be based on internal doses (Partosch *et al.*, 2015).

3-14 ASPECTS TO CONSIDER WITH RESPECT TO THE RISK ASSESSMENT FOR THE INHALATION ROUTE

For a number of cosmetic products, inhalation is also a potential exposure route. This is the case for instance when substances with a low boiling point and high vapour pressure are used, like solvents in *e.g.* a nail polish remover. Other examples are the increasing use of cosmetic products in spray form, like deodorants and hair sprays, but also sunscreens, and cosmetic powders such as face powder. For these products, the primary dermal exposure has to be evaluated (Steiling *et al.*, 2012), but in case exposure via inhalation is possible, this has to be also taken into account in the risk assessment for both possible local effects as well as potential internal exposure.

For appropriate assessment of the toxicity via inhalation, knowledge of the hazard profile of the cosmetic ingredients, their concentrations in the final product as well as the likely exposure scenario of the final product is needed. Overviews on the evaluation of the safety of cosmetic substances in spray products are given by Rothe *et al.* (2011) and Steiling *et al.* (2014). In Fig. 4, the basic principles for the safety evaluation of inhalable cosmetic products and their ingredients are provided.

3-14.1 Hazard assessment for the inhalation route

a) Local respiratory tract toxicity

The toxic effects of a chemical on the respiratory tract can be assessed based on an inhalation study. Information on irritancy to eyes and mucous membranes (*in vivo* and *in vitro*) could also be useful in regard to local effects in the respiratory tract.

b) Systemic toxicity

In a standard toxicological dossier for a cosmetic ingredient, there are generally no data available with regard to the inhalation route, although an acute inhalation toxicity (LC_{50}) study or an inhalation study with repeated exposure might be provided.

c) In vitro methods

A number of human-based reconstructed tissue co-culture cell 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). Information from these models could however be used as supportive information on inhalation toxicity.

3-14.2 Exposure assessment for the inhalation route

During use, a spray product may be released as a vapour or as an aerosol. For exposure to aerosols, the size of the airborne particles/droplets to which the consumer is exposed determines the extent of the inhalation exposure. A sprayed formulation generally consists of droplets of different sizes and/or particles which may undergo ageing and evaporation of solvent before they reach the airways. The fraction comprising droplets/particles with a Mass Median Aerodynamic Diameter (MMAD) of ≤100 µm is generally regarded inhalable. Usually, three main fractions of the airborne aerosol are distinguished: the inhalable fraction, the thoracic fraction, and the respirable fraction. These particle size fractions are defined in the EU-standard EN 481 for measurements in work places (CEN, 1993).

Thus, whereas particles/droplets with a MMAD <100 µm can reach the nose and the mouth, particles/droplets >10 µm are generally retained in the nose, mouth, throat or tracheobronchial area. After mucociliary clearance, further intake of insoluble particles or their components via the oral route may occur in humans. Taking also mouth breathing of humans into account, only particles/droplets with a MMAD <10 µm are small enough to reach the deeper part of the human trachea and the lungs, where they can enter the alveoli

and may become systemically available (Snipes, 1989; Valentine and Kennedy, 2008). This is different from laboratory animals where only particles with a MMAD < 1 to 5 µm are capable of reaching the lung.

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/droplet size <10 µm, compared to pump sprays, which in general produce larger particles/droplets. However, also for pump sprays the size of the droplets produced depends on the spray nozzle and studies *e.g.* by Quadros and Marr (2011), and Losert *et al.* (2015) have shown that pump sprays can also produce particles/droplets in the nano size range. Another important consideration in relation to the airborne droplets/particles is that they can dry off quickly while airborne and become small enough to become respirable due to evaporation of the solvents/ formulants. It is therefore recommended that safety assessment of the sprayable products should take into account not only size distribution of the generated aerosol droplets but also their size distribution just before settling. This is especially important for spray/sprayable cosmetic products containing nanomaterials, for which measured droplet size as well as size distribution of the dried residual particles will need to be provided.

The size of the droplets after spraying in a spray formulation is influenced by the actual formulation (surface tension) and by the different solvents and propellants used in the formulation. They are also well related to the geometry of the spray nozzle and the can size. Information on the realistic particle/droplet 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. An important consideration in relation to the airborne droplets/particles is that they can dry off quickly while airborne and become small enough to become respirable due to evaporation of the solvents/ formulants. It is therefore recommended that safety assessment of the sprayable products should take into account not only size distribution of the generated aerosol droplets but also their size distribution before settling. See Section 4-3.5.

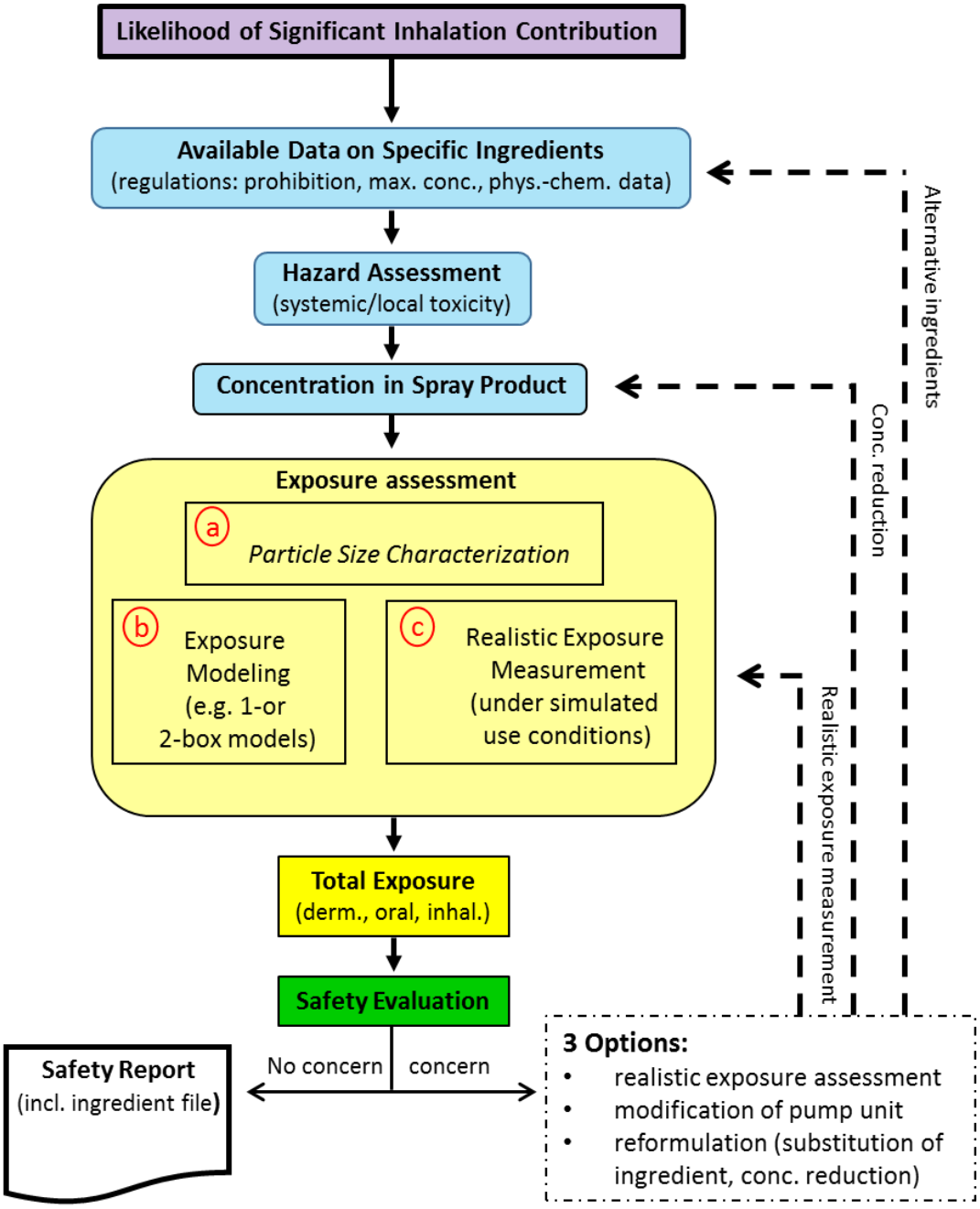

Fig. 4: Basic principles for the safety assessment of inhalable cosmetic products and their ingredients.

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). Default equations can be used as a conservative, worst case approach, and as a first estimate (ECHA, 2012b). For a more realistic assessment, higher tier models like the ConsExpo model can be considered (RIVM, 2012).

For sprayable products, see Section 4-3.5.

4. SAFETY EVALUATION OF FINISHED COSMETIC PRODUCTS

4-1 INTRODUCTION

The most significant changes for finished cosmetic products introduced by the new Cosmetics Regulation were mentioned in Section 3-1 and can be summarised as follow:

1. **Strengthened safety requirements for cosmetic products**
   Manufacturers need to follow specific requirements in the preparation of a product safety report prior to placing a product on the market.

2. **Introduction of the notion of "responsible person"**
   Only cosmetic products for which a legal or natural person is designated within the EU as a "responsible person" can be placed on the market. The new Cosmetics Regulation allows the precise identification of the responsible person and clearly outlines their obligations.

3. **Centralised notification of all cosmetic products placed on the EU market**
   Manufacturers will need to notify their products only once – via the EU CPNP.

4. **Introduction of reporting of serious undesirable effects (SUE)**
   A responsible person will have an obligation to notify SUE effects to national authorities. The authorities will also collect information coming from users, health professionals, and others. They will be obliged to share the information with other EU countries.

5. **New rules for the use of nanomaterials in cosmetic products**
   Colourants, preservatives and UV-filters, including those that are nanomaterials, must be explicitly authorised. Products containing other nanomaterials not otherwise restricted by the Cosmetics Regulation will also be the object of a full safety assessment at EU level if the Commission has safety concerns. Nanomaterials used in cosmetic products must be labelled in the list of ingredients with the word "nano" in brackets following the name of the substance, *e.g.* "titanium dioxide (nano)".

In accordance with the requirements of Regulation (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 (CPSR: Cosmetic Product Safety Report), made by a safety assessor, with the competences as required Art. 10.2. 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 (2013/674/EU).

It must be emphasised that it remains the ultimate responsibility of the responsible person (via 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 diluted 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 3-4.9),
- 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 3-11).

Every specific exposure scenario will be linked to a certain amount of a 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 (see Section 3-12).

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 sensitisation 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 result, among other things, in the determination of the SED, an important parameter for calculating the MoS of substances in a finished cosmetic product [MoS = NOAEL / SED].

The following calculations take into account the **dermal** exposure of the cosmetic product ingredients under consideration. 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/d) = Systemic Exposure Dose
- DA_a (µg/cm²) = Dermal Absorption reported as amount/cm², resulting from an assay under in-use mimicking conditions[1]
- SSA (cm²) = Skin Surface Area expected to be treated with the finished cosmetic product (see Section 4-2 for SSA values per product type)
- F (day^{-1}) = Frequency of application of the finished product (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 1 are extracted from a Dutch study on cosmetic exposure assessment performed by the RIVM (Bremmer *et al.*, 2006a, 2006b) and indicate exposed skin surface areas per cosmetic product type[2]. The last column of the same table reflects the presumed **frequency of application (F)** of the finished product.

Table 1: Mean exposed skin surface area per product type (Bremmer *et al.*, 2006a, 2006b) 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[3]
Bath oil, salts, etc.	16340	area body - area head	1/day
Hair care			
Shampoo	1440	area hands + 1/2 area head	1/day

[1] In case the *in vitro* dermal absorption assay was not performed under in-use conditions, an additional correction factor can be introduced.
[2] 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).
[3] Danish Ministry of the Environment, Environmental Protection Agency: Survey of liquid hand soaps, including health and environmental assessments.

Product type	Skin surface area involved (RIVM)		Frequency of application*
	Surface area (cm²)	Parameters (if specified)	
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[1]		*2/day*
Deodorant/antiperspirant			
Deodorant aerosol spray[2] and non-spray[3]	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 correspond to the 90th percentile values of the 2005/2009 Cosmetics Europe studies (see furher 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:

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

[1] Ferrario *et al.*, 2000.
[2] Steiling *et al.*, 2012
[3] Cowan-Ellsberry *et al.*, 2008.

With:	SED (mg/kg bw/d) =	Systemic Exposure Dose
	A (mg/kg bw/d) =	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 2
	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[1]

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 Cosmetics Europe. 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).

The figures for the daily consumed amounts of cosmetic products (measured by weight) are taken up here. In the Cosmetics Europe studies, it was shown that for many product types there is often an **inverse relationship 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 not appropriate to calculate daily exposure by simple multiplication of the maximum frequency per day value by the maximum quantity per application value.

Therefore, Table 2 displays the daily amount applied and the retention factor[2] to come to the final daily dermal exposure to the finished product. For the product types included in the recent 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 wants to know the average use frequency related to the obtained data, reference is made to Table 1, which displays skin surface area involved, and also the assumed frequency of use.

Another feature in the calculation, and important for Table 2, is the fact that the body weight is already incorporated in the daily amount of product applied. This accounts for the 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 2 correspond to the 90th percentile[3]. In case of product types for which such data was not available, the 'old' application value (as given in the 8th Revision of the SCCS Notes of Guidance) was divided by the mean human body weight of 60 kg.

[1] In case the *in vitro* dermal absorption assay was not performed under in-use conditions, an additional correction factor can be introduced.
[2] 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)
[3] 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.

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.

Recent studies (Biesterbos *et al.*, 2013; Ficheux *et al.*, 2014, 2015), provide exposure values for a series of cosmetic products. These values have not been taken up here, since they have been conducted on a more limited scale than the Cosmetics Europe studies and provide a restricted population diversity (only Dutch and French population, respectively). As the study results in general show lower exposure values than those reported in the Cosmetics Europe studies, they are considered less protective for human health and therefore most of the data have not been taken up in the following Tables. The results regarding nail polishes and nail polish removers of both studies are similar and are listed in Table 3.

Table 2: Estimated daily exposure levels for different cosmetic product types according to Cosmetics Europe data (SCCNFP/0321/00; Hall *et al.*, 2007, 2011).

Product type	Estimated daily amount applied	Relative amount applied (mg/kg bw/d)	Retention factor [1]	Calculated daily exposure (g/d)	Calculated relative daily exposure (mg/kg bw/d)
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	0.69 g	10.00	1.0	0.69	10.00

[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 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.,* 2014: 'Ethanol-based' are products containing ethanol as principal ingredient.

Product type	Estimated daily amount applied	Relative amount applied (mg/kg bw/d)	Retention factor [1]	Calculated daily exposure (g/d)	Calculated relative daily exposure (mg/kg bw/d)
(not ethanol-based)					
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

Table 3: Estimated exposure levels for nail polishes and nail polish removers (Biesterbos *et al.*, 2013; Ficheux *et al.*, 2014).

Product type	Mean amount/application		Mean amount/day	
	Biesterbos *et al.* 2013	Ficheux *et al.*; 2014	Biesterbos *et al.* 2013	Ficheux *et al.*; 2014
Nail polish	0.3 g	0.3 g	0.04 g	0.05 g*
Nail polish remover	2.0 ml	2.7 g	0.3 ml	0.45 g*

* Based on mean frequency of use per week in different age categories: mean frequency of use is 1.17 per week (Ficheux et al., 2014).

For **sunscreen lotion**, an application of **18.0 g/d** is used in the MoS calculation. It is used in risk assessment, but is not meant as a recommended amount to be applied by the consumer (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 reported (Stenberg *et al.*, 1985; Bech-Thomsen *et al.*, 1993; Diffey, 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 hurriedly self-applied or applied to both hairy skin and areas which are difficult to reach such as the back and the lower legs.

For some cosmetic substances, individual product type exposure values as mentioned in Table 2 might not reflect the overall exposure to these compounds, since there is a clear possibility that 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 mostly 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.

Recently, however, aggregate exposure to UV-filters due to their presence in common cosmetics has been mentioned to be a possible parameter that could affect the MoS-value of some UV-filters used in sunscreen products (Manová *et al.*, 2013). Only scarce data are available for the Swiss population. The most commonly used UV-filters for daily protection are butyl methoxydibenzoylmethane and ethylhexyl methoxycinnamate (Manová *et al.*, 2013). In order to see whether the amounts reported affect the safe use of these filters as sunscreens, MoS calculations were carried out for aggregate exposure of both filters and MoS-values of > 100 were obtained, suggesting that the use of these UV-filters in sun protection products is safe, despite their occurrence in a variety of other cosmetic products.

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

Type of exposure	Product	g/d	mg/kg bw/d
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 5 and the inhalation risk is 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 in Section 3-14.

4-3 GUIDELINES FOR THE SAFETY EVALUATION OF FINISHED COSMETIC PRODUCTS

4-3.1 Introduction

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.) and cases where 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 (*in silico* data);
- relevant data on analogous compounds.

The general toxicological requirements for cosmetic substances have been described in detail in Section 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 biologically relevant 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 NOAEL value is available for a specific substance, its 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 verifiable independence in matters of product-related decisions.

4-3.5 Safety assessment of sprayable products

For sprayable products, the "likelihood of significant inhalation contribution" (Steiling, 2014) cannot be ruled out. Therefore, a safety assessment is needed.
The term 'spray' or 'sprayable' means that a formulation is either dispensed by the use of propellant gas as defined in Directive 75/324 (**propellant spray**), or by a spray bottle with a pump dispenser that forces a liquid through a nozzle generating a spray stream or a mist of a liquid (**pump spray**) (SCCS/1539/14). For safety assessment concerning local effects, the modelled or measured local consumer exposure to the sprayed cosmetic ingredient/product is compared with the dose considered to be without any local toxicological adverse effect based on the outcome of standard toxicological tests. For safety assessment regarding systemic effects, the internal dose needs to be calculated and added to the dose received from other intake routes. This total dose (Fig. 4) is then compared to the most sensitive systemic 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, 2012a). 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. See also Section 3-14.

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 with compromised immune system. 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.

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

[1] Colipa is now called "Cosmetics Europe"

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 2014, European Pharmacopoeia 2014)

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, Candida albicans* and *Aspergillus brasiliensis.*

It is well known today 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 microbicidal activity of preservatives or any other compound in the finished cosmetic must be ruled out in the challenge test by dilution, filtration, 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 or universal challenge test method is currently available, it is up to the responsible person to decide on the details of the test to be used.

4-4.4 Good Manufacturing Practice (GMP)

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 European Committee for standardization (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

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EC B.46 – *In vitro* skin irritation: Reconstructed human epidermis test method Commission Regulation (EU) No 640/2012 of 6 July 2012 **amending,** for the purpose of its adaptation to technical progress, Regulation (EC) No 440/2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). *Official Journal L 193, 20/07/2012, p. 17.*

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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[1]. 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 GROW (Directorate-General for Internal Market, Industry, Entrepreneurship and SMEs) has built up a free to use database of cosmetic substances called **CosIng**, http://ec.europa.eu/consumers/cosmetics/cosing/ (Cosmetic ingredients) which combines INCI names and synonyms of the listed substances with useful regulatory information. CosIng database is regularly updated with information on new cosmetics ingredients.

Finally, this section 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 3.7). 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 (CINs) 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.

[1] For references, see No. 6 of this Appendix

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 colourants a colour index (CI) number
- Chemical name
- Chemical Abstract Service (CAS) number
- 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, the 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 has 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 2015

APPENDIX 2: STANDARD FORMAT OF THE OPINIONS

SCCS/XXXX/year

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 that 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 independent 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

……..XXXXXXXX (names)

Contact
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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 SCCS and of the concerned working group, with identification of chair and rapporteur(s).

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List of member(s) of the other scientific committee (if applicable):
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List of external experts (if applicable):

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(If relevant: This Opinion has been subject to a commenting period of minimum four weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged. Revised opinions carry the date of revision.)

Keywords: ……; ……; ……; ……; ……

Opinion to be cited as: ……
……

TABLE OF CONTENTS

1. BACKGROUND

2. TERMS OF REFERENCE

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

Ref.:

3.1.1.2 Chemical names

Ref.:

3.1.1.3 Trade names and abbreviations

Ref.:

3.1.1.4 CAS / EC number

Ref.:

3.1.1.5 Structural formula

Ref.:

3.1.1.6 Empirical formula

Ref.:

3.1.2 Physical form

Ref.:

3.1.3 Molecular weight

Ref.:

3.1.4 Purity, composition and substance codes

Ref.:

3.1.5 Impurities / accompanying contaminants

Ref.:

3.1.6 Solubility

Ref.:

3.1.7 Partition coefficient (Log P_{ow})

Ref.:

3.1.8 Additional physical and chemical specifications

Where relevant:

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

Ref.:

3.1.9 Stability

Ref.:

3.2 FUNCTION AND USES

Ref.:

3.3 TOXICOLOGICAL EVALUATION

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

Ref.:

3.3.1.2 Acute dermal toxicity

Ref.:

3.3.1.3 Acute inhalation toxicity

Ref.:

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

Ref.:

3.3.2.2 Mucous membrane irritation/eye irritation

Ref.:

3.3.3 Skin sensitisation

Ref.:

3.3.4 Dermal / percutaneous absorption

Ref.:

3.3.5 Repeated dose toxicity

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

Ref.:

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

Ref.:

3.3.5.3 Chronic (> 12 months) toxicity

Ref.:

3.3.6 Reproductive toxicity

3.3.6.1 Fertility and reproduction toxicity

Ref.:

3.3.6.2 Developmental toxicity

Ref.:

3.3.7 Mutagenicity / genotoxicity

3.3.7.1 Mutagenicity / genotoxicity *in vitro*

Ref.:

3.3.7.2 Mutagenicity / genotoxicity *in vivo*

Ref.:

3.3.8 Carcinogenicity

Ref.:

3.3.9 Toxicokinetics

Ref.:

3.3.10 Photo-induced toxicity

3.3.10.1 Phototoxicity/photoirritation and photosensitisation

Ref.:

3.3.10.2 Phototoxicity / photomutagenicity / photoclastogenicity

Ref.:

3.3.11 Human data

Ref.:

3.3.12 Special investigations

Ref.:

3.3.13 Safety evaluation (including calculation of the MoS)

Ref.:

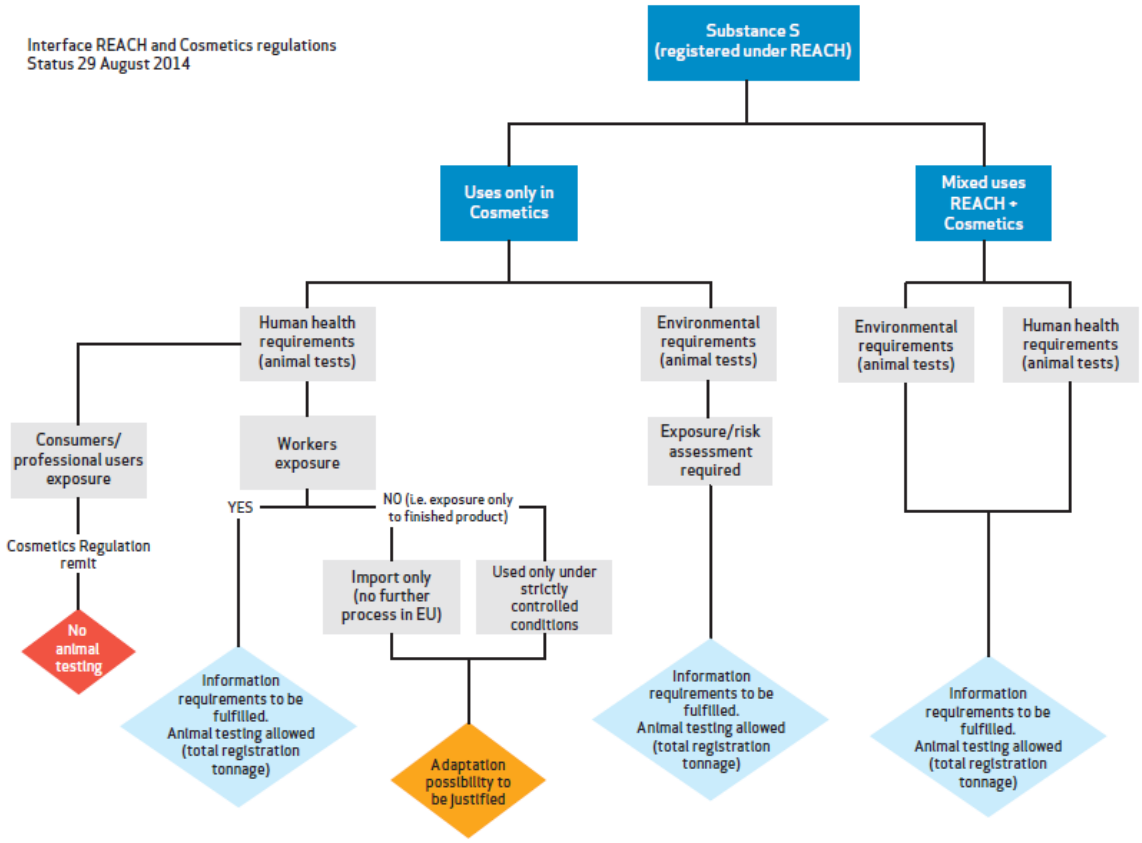
3.3.14 Discussion

4. CONCLUSION

5. MINORITY OPINION

6. REFERENCES

APPENDIX 3: ANIMAL TESTING: INTERFACE BETWEEN REACH AND COSMETICS REGULATIONS



Reference: Interface between REACH and Cosmetics regulations (ECHA, 2014a)

APPENDIX 4: CONCEPTUAL FRAMEWORK FOR TESTING AND ASSESSMENT OF ENDOCRINE DISRUPTERS[1]

The Conceptual Framework lists the OECD TGs and standardized test methods available, under development or proposed that can be used to evaluate chemicals for endocrine disruption. The Conceptual Framework is intended to provide a guide to the tests available which can provide information for endocrine disruptors' assessment but is not intended to be a testing strategy. Furthermore, this Conceptual Framework does not include evaluation of exposure, however, this should be included when deciding whether further testing is needed. Further information regarding the use and interpretation of these tests is available in GD 150 *(OECD 2012a*).

Mammalian Toxicology

Level 1: Existing data and non-test information
- Physical & chemical properties, *e.g.* MW reactivity, volatility, biodegradability
- All available toxicological data from standardized or non-standardized tests.
- Read across, chemical categories, QSARs and other *in silico* predictions, and ADME model predictions

Level 2: *In vitro* assays providing data about selected endocrine mechanism(s) / pathways(s) (Mammalian and non-mammalian methods)
- Estrogen or androgen receptor binding affinity
- Estrogen receptor transactivation (OECD TG 455 and TG 457)
- Androgen or thyroid transactivation (If/when TGs are available)
- Steroidogenesis *in vitro* (OECD TG 456)
- MCF-7 cell proliferation assays (ER ant/agonist)
- Other assays as appropriate

Level 3: *In vivo* assays providing data about selected endocrine mechanism(s) / pathway(s) [2]
- Uterotrophic assay (OECD TG 440)
- Hershberger assay (OECD TG 441)

Level 4: *In vivo* assays providing data on adverse effects on endocrine relevant endpoints [3]
- Repeated dose 28-day study (OECD TG 407)
- Repeated dose 90-day study (OECD TG 408)
- 1-generation reproduction toxicity study (OECD TG 415)
- Male pubertal assay (see GD 150 Chapter C4.3) [4]
- Female pubertal assay (see GD 150 Chapter C4.4) [4]
- Intact adult male endocrine screening assay (see GD 150 Chapter Annex 2.5)
- Prenatal developmental toxicity study (OECD TG 414)
- Chronic toxicity and carcinogenicity studies (OECD TG 451-452-453)

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[1] Text on the OECD Conceptual framework **as** cited **in EFSA 2013,** Annex C, p. 65, with minor modifications. Excerpted from OECD (2012a) Guidance document on standardized test guidelines for evaluating chemicals for endocrine disruption. Series on Testing and Assessment no. 150, ENV/JM/MONO(2012)22 http://search.oecd.org/officialdocuments/displaydocumentpdf/?cote=env/jm/mono%282012%2922&doclanguage=en (OECD, 2012a), pp. 385-387 – consulted September 2015

(Numbering of footnotes in part modified as only mammalian tests are listed here)

[2] Some assays may also provide some evidence of adverse effects.

[3] Effects can be sensitive to more than one mechanism and may be due to non-ED mechanisms.

[4] Depending on the guideline/protocol used, the fact that a substance may interact with a hormone system in these assays does not necessarily mean that when the substance is used it will cause adverse effects in humans or ecological systems.

- Reproductive screening test (OECD TG 421 if enhanced)
- Combined 28-day/reproductive screening assay (OECD TG 422 if enhanced)
- Developmental neurotoxicity (OECD TG 426)

Level 5: *In vivo* assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism [3]
- Extended one-generation reproductive toxicity study (OECD TG 443) [5]
- 2-Generation reproduction toxicity study (OECD TG 416 most recent update)

(Numbering of footnotes in part modified as only mammalian tests are listed here)
[3] Effects can be sensitive to more than one mechanism and may be due to non-ED mechanisms.
[5] The new EOGRT study (OECD TG 443) is preferable for detecting endocrine disruption because it provides an evaluation of a number of endocrine endpoints in the juvenile and adult F1, which are not included in the 2-generation study (OECD TG 416) adopted in 2001

APPENDIX 5: CMR GUIDANCE ON SAFE USE OF CMR SUBSTANCES IN COSMETIC PRODUCTS

GUIDANCE ON A HARMONISED APPROACH TO THE DEVELOPMENT AND USE OF OVERALL EXPOSURE ESTIMATES IN ASSESSING THE SAFE USE OF CMR SUBSTANCES IN COSMETIC PRODUCTS

I. Background

1. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products[1] (Cosmetics Regulation) contains in its Article 15 provisions on the use in cosmetic products of substances classified as carcinogenic, mutagenic or toxic for reproduction (CMR substances) under Part 3 of Annex VI to Regulation (EC) 1272/2008[2]. These provisions apply from 1 December 2010.

2. As a general rule, the substances classified as CMR substances of category 1A, 1B and 2 under Part 3 of Annex VI to Regulation (EC) 1272/2008 are prohibited for use in cosmetic products. This ban is automatic as from the date of application of their classification under Regulation (EC) No 1272/2008.

3. However, exceptions to this rule are foreseen by the Cosmetics Regulation. Indeed, a substance classified as a CMR substance of category 2 may be used in cosmetic products where the substance has been evaluated by the Scientific Committee on Consumer Safety (SCCS) and found safe for use in cosmetic products on the basis of the data submitted.

4. Also, CMR substances of category 1A or 1B may be used in cosmetic products by way of exception where, subsequent to their classification as CMR substances of category 1A or 1B under Part 3 of Annex VI to Regulation (EC) No 1272/2008, all of the following conditions are fulfilled:

(a) they comply with the food safety requirements as defined in Regulation (EC) No 178/2002 of the European Parliament and the Council of 28 January 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;[3]

(b) there are no suitable alternative substances available, as documented in an analysis of alternatives;
(c) the application is made for a particular use of the product category with a known exposure; and
(d) they have been 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.

II. Scope and objectives

5. Article 15, paragraph 3 of the Cosmetics Regulation foresees that the Commission shall ensure that appropriate guidance is developed with the aim of enabling a

[1] OJ L 342, 22.12.2009, p. 59.
[2] OJ L 353, 31.12.2008, p. 1.
[3] OJ L 31, 1.2.2002, p. 1.

harmonised approach to the development and use of overall exposure estimates in assessing the safe use of CMR substances.

6. To authorise the use of CMR substances of category 1A or 1B in cosmetic products, one of the conditions to be fulfilled is that they have been evaluated and found safe by the SCCS for use in cosmetic products, in particular in view of exposure to cosmetics products and taking into consideration the overall exposure from other sources and vulnerable population subgroups.

7 On a case by case basis and at the request of the SCCS, it may also be necessary to perform an overall exposure from other sources for CMR 2 substances. Therefore the procedure developed below for the overall exposure assessment of CMR 1A and 1 B substances should, where necessary, also apply to CMR 2 substances (condition (d) only).

8. Appropriate consultations with the SCCS and other relevant stakeholders have been carried out in order to develop this guidance. In addition, administrative agreements have been established with relevant EU Agencies - European Chemicals Agency (ECHA), European Food Safety Authority (EFSA), European Medicines Agency (EMA) - to ensure the appropriate exchange of data between them and the SCCS Secretariat.

III. Procedure

9. The aim of this guidance is to outline the mechanisms necessary for ensuring the generation and the exchange of the appropriate data for the assessment by the SCCS of the overall exposure to a CMR 1A or 1B substance stemming from other sources than cosmetics (such as food, biocides, etc.).

10. When a substance of interest for the industry is indicated in the Registry of Intentions for the purpose of its harmonised classification as CMR substance under Part 3 of Annex VI to Regulation (EC) No 1272/2008, it is for the industry to inform the Commission in due time of its intention to defend a substance under discussion for future (re)classification as CMR substance, so as to allow that any possible derogation measure is adopted by the Commission within 15 months following the adoption of the classification as CMR substance.

11. The Commission responsible Services should inform the SCCS that the industry intends to defend the substance. They should also inform the Member States of this intention, so that any relevant data available in public or state laboratories, or elsewhere, may be considered for the scientific assessment. In parallel, they may also organise a call for scientific data from anyone holding or being aware of further relevant information, in order to gather additional scientific data.

12. It is the industry's responsibility to demonstrate that the first three conditions (a), (b) and (c) for derogation laid down in Article 15 paragraph 2 of Cosmetics Regulation are fulfilled. For justifying compliance with each of the above conditions, the industry should submit appropriate dossiers for examination by the Commission responsible Services.

13. The Commission responsible Services should verify the compliance with the food safety requirements, where necessary by consulting the EFSA and the absence of suitable alternative substances and the fact that the application is limited for a particular use of the product category with a known exposure, where necessary by consulting the COSCOM.

14. Subsequently, the procedure for the exchanges of data between the relevant entities can be started as regards to the overall exposure assessment by the SCCS (condition d). Requests for data sharing with the relevant EU Agencies (ECHA, EFSA and EMA[4]) should be initiated and managed by the SCCS Secretariat. On a case by case basis, the Commission responsible Services can, where relevant, ask for data to Member States or third countries.

15. The "Declaration of Commitment by the Commission with respect to security aspects for ECHA's information systems" has been signed by the responsible Commission Services[5] and sets up the conditions under which exchange of confidential data from REACH dossiers can be ensured with ECHA.

16. Upon request by the SCCS Secretariat, the Commission responsible Services should grant access to relevant data in REACH registration dossiers to a designated SCCS expert who adheres to the security rules for users of ECHA's Information System.

17. The extraction of relevant data from REACH dossiers and their processing to establish aggregated exposure levels should be completed by the designated SCCS expert within the secure room of the Commission responsible Services and in accordance with all applicable security rules. In case an evaluation of the CMR substance has already been completed under REACH, exposure levels that have been established can also be used straightaway where appropriate.

18. The EFSA should be consulted by the SCCS Secretariat to provide, if available, data or estimates on exposure from food and other relevant sources.

19. Additionally, the EMA could be consulted by the SCCS Secretariat on a case by case basis on exposure from substances used as pharmaceuticals.

20. The applicant should include in their submission all exposure information they have. In addition to the exposure information gathered as mentioned above, *e.g.* exchange of data with the Agencies, public call for information, consultation with Member States, the SCCS will consider the exposure information provided by the applicant.

21. It is necessary that the exchange of data takes place in a smooth and timely manner as, for CMR 1A and 1B substances, the measure necessary for the derogation must be adopted by the Commission within 15 months starting from listing of the substance in Part 3 of Annex VI to Regulation (EC) No 1272/2008.

22. The SCCS, once it has received the scientific data from ECHA, EFSA, EMA and has taken into consideration the data submitted by the industry and other available sources (such as information gathered from Member States or following public consultation), shall assess the specific CMR substance(s) for safety of use in cosmetic products taking into account the overall exposure from other sources and vulnerable population groups within a timescale of at least six months for finalising their Opinion after an adequate submission and a complete set of exposure data is received.

[4] The need to consult EMA will be checked by the Commission on a case by case basis.
[5] DG ENTR and DG ENV co-managed the REACH legislation.

23. It should be noted that, where the work of other scientific/regulatory bodies contains information on exposure to humans via the environment, this may have been incorporated in their overall estimates of exposure. However, Cosmetic Regulation (EC) No 1223/2009 only covers the aspects of safety to human health. As indicated in recital 5 of that Regulation, the environmental concerns that substances used in cosmetic products may raise are considered through the application of Regulation (EC) No 1907/2006 (REACH).[6]

24. As regards the scientific risk assessment of CMR substances of categories 1A and 1B used in cosmetics, the SCCS will determine the most appropriate methodology for their safety evaluation based on the best scientific knowledge and taking into account the exposure from the specific uses in cosmetic products and the overall exposure from other sources.

25. In order to provide transparency on the applied methodology and guidance to the industry, the SCCS should develop and incorporate this methodology within the next revision of its "Notes of guidance[7] for the testing of cosmetic substances and their safety evaluation".

IV. Final observations

26. This document is only meant to provide guidance for a harmonised approach to the development and use of overall exposure estimates in assessing the safe use of CMR substances in cosmetic products and it is by no means binding.

27. The SCCS evaluation will not automatically trigger action under any legislation other than the Cosmetics legislation. The SCCS conclusions will be publicly available.

28. This document may be revised in the future in the light of further scientific developments.

[6] OJ L 396, 30.12.2006, p. 1.
[7] SCCS/1501/2 of 11 December 2012.

ABBREVIATIONS AND GLOSSARY OF TERMS

3D	Three-dimensional
3R	Refinement, Reduction, Replacement
3T3 NRU PT	3T3 Neutral Red Uptake Phototoxicity Test
A[1]	Estimated daily exposure amount per kg body weight used in calculation of SED (%)
ADME	Absorption, distribution, metabolism, excretion
Adverse	An adverse response is defined as any treatment-related response that results in change in the morphology, physiology, growth, development or life span of an organism, which results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other environmental influences (WHO 2004)
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; Russell *et al.,* 1959)
AOP	Adverse outcome pathway
Art.	Article
ATP	Adenosine Triphosphate
BCOP	Bovine Corneal Opacity and Permeability
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.
BrdU	5-bromo-2-deoxy-uridine
BSE	Bovine Spongiform Encephalopathy
BW	Body Weight
CAS n°	Chemical Abstracts Service registry number
Cat.	Category
CEN	European Committee for Standardization
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
CM	Cytosensor Microphysiometer test method

[1] Used in the calculation of the Systemic Exposure Dose (see Section 3-12.2)

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)
CPSR	Cosmetic Product Safety Report
CPNP	Cosmetic Products Notification Portal
CTA	Cell Transformation Assay
CYP	Human Cytochrome P450
C (%)[1]	Concentration of the substance in finished cosmetic product
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 ENV	Directorate-General Environment
DG GROW (ENTR)	Directorate-General Growth
DG SANTE (SANCO)	Directorate-General Health and Food safety

[1] Used in the calculation of the Systemic Exposure Dose (see Section 3-12.2).

DHA	Dihydroxyacetone
Dir.	Directive
DNA	DeoxyriboNucleic Acid
Doc.	Document
Dose	Total amount of an agent administered to, taken up by, or absorbed by an organism, system, or (sub)population (WHO 2004). 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 such as NOAEL, LOAEL, BMD, T25 etc. (ECHA, 2012a)
DPRA	Direct Peptide Reactivity Assay
EC	European Community
EC Number	EC number, meaning either EINECS number, ELINCS number, NLP number or EC Number appointed by the European Commission under REACH Regulation The European Community number (EC Number) is a unique seven-digit identifier that was assigned to substances for regulatory purposes within the European Union by the European Commission. The so-called EC Inventory comprises three individual inventories, EINECS, ELINCS and the NLP list.(1). (ECHA) also applies the EC number format to what it calls "List number".[6] The number are assigned under the REACH Regulation without being legally recognised. Hence, they are not official because they have not been published in the *Official Journal* of the European Union. List numbers are administrative tools only and shall not be used for any official purposes.
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals ECETOC is an industry-funded expert not-for-profit think tank whose sole purpose is to enhance the quality of chemicals risk assessment so that chemicals management decisions are informed, reliable and safe.
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
EIT	Eye Irritation Test
ELINCS	European List of Notified Chemical Substances
ELISA	Enzyme-Linked Immunosorbent Assay
EMA/EMEA	European Medicines Agency
EOGRTS	Extended One-Generation Reproductive Toxicity Study

(US) EPA	(United States) Environmental Protection Agency
ESAC	ECVAM Scientific Advisory Committee
EST	Embryonic Stem cell Test
EU	European Union
EURL-ECVAM	European Union Reference Laboratory - European Centre for the Validation of Alternative Methods
F	Frequency of application
FDA	Food and Drug Administration (federal agency of the United States Department of Health and Human Services)
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)
FL	Fluorescein Leakage test
GC-MS	Gas Chromatography–Mass Spectrometry
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
HPLC	High-Performance Liquid Chromatography
HPLC-PDA	High-Performance Liquid Chromatography/Photo-Diode Array detection
HPRT	Hypoxanthine-guanine PhosphoRibosyl Transferase
HT25	Human dose-descriptor, derived from T25 and based on comparative metabolic rates (Sanner *et al.,* 2001)
IARC	International Agency for Research on Cancer
IATA	Integrated Approaches to Testing and Assessment
ICCR	International Cooperation on Cosmetics Regulation
ICE	Isolated Chicken Eye
***In silico* methods**	Computational approaches that use (quantitative) structure-activity relationship modelling, and read-across between substances on the basis of structural or functional similarities (ICCR, 2014)
***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
IL-1α	Interleukin-1α
INN	International Non-proprietary Name
IPCS	International Programme on Chemical Safety
IR	Infrared Spectroscopy
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 2009b).
LC-MS	Liquid Chromatography–Mass Spectrometry
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.1 bis)
LED	Lowest Effective Dose, *e.g.* LED10
LLNA	Local Lymph Node Assay
LO(A)EL	The Lowest Observed (Adverse) Effect Level is the outcome of repeat-dose 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 dose where (adverse) effects can be observed. In the calculation of the MoS, the lowest obtained LOAEL value may be used when a NOAEL is not available. The LOAEL should be expressed as mg/kg bw/d. (ECB, 2003)
MDCK	Madin-Darby canine kidney cells
MIE	Molecular Initiating Event
MM	MicroMass
MMAD	Mass Median Aerodynamic Diameter
MN	MicroNucleus
MoE	Margin of Exposure
MoS	Margin of Safety
MR	Mitotic Recombination
MS	Mass Spectrometry
MTT	3-(4,5)-dimethyl-2-thiazolyl-2,5-dimethyl-2H-tetrazolium bromide
MW	Molecular Weight
Nanomaterial	An insoluble or bio-persistent 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). Deviating definitions in other regulatory fields may also exist.
NAT1	N-acetyltransferase 1
NLP	No Longer Polymer
NMR	Nuclear Magnetic Resonance
NOAEC	No observable adverse effect concentration

NO(A)EL, NO(A)EL$_{sys}$	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 dose for which no (adverse) effects can be observed (based on EC B.26). The NOAEL should be expressed as mg/kg bw/d. In the calculation of the MoS, the lowest obtained NOAEL value is used, in order to take into account the most sensitive species, as well as the relevant effect occurring at the lowest dose possible. Whereas the NOAEL is a dose descriptor for an external dose, the **NOAEL$_{sys}$** is a dose descriptor of the systemic exposure to a substance and is calculated from the NOAEL by use of the proportion of the substance systemically absorbed.
NRU	Neutral Red Uptake
NTP	National Toxicology Program
OD	Optical Density
OECD	Organisation for Economic Co-operation and Development
PBMDC	Peripheral Blood Monocyte Derived dendritic Cells
PBPK	Physiologically based pharmacokinetics
PBPK modelling	Physiologically based pharmacokinetic modelling
PBTK	Physiologically based toxicokinetics
PBTK modelling	Physiologically based toxicokinetic modelling
PIF	Product Information File
P$_{ow}$	n-octanol / water partition coefficient
PPD	p-Phenylenediamine
ppm	parts per million (*e.g.* mg/kg)
PPRA	Peroxidase Peptide Reactivity Assay
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)
QRA	Quantitative Risk Assessment
QSAR	Quantitative Structure-Activity Relationship
REACH	Registration, Evaluation, Authorisation and restriction of Chemicals
Reference material	Material sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process (ISO, 2008).
RhCE	Reconstructed human Cornea-like Epithelium test method
RhE	Reconstructed Human Epidermis
RIVM	Rijks Instituut voor Volksgezondheid en Milieu
rLLNA	reduced Local Lymph Node Assay
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
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 Dose of a cosmetic ingredient is the amount expected to enter the blood stream (and therefore be systemically available) per kg body weight and per day. It is expressed in mg/kg body weight/day. For this definition a mean human body weight of 60 kg is commonly accepted. Since the majority of cosmetic products are applied topically, systemic availability will strongly depend on the dermal absorption of the compound. This can be determined according to the tests described in Section 3-4.1.1. Nevertheless, the results of these tests can be interpreted in two different ways (see Section 3-12.2: dermal absorption issues).
SHE	Syrian Hamster Embryo
SIT	Skin Irritation Test
Spray, sprayable cosmetic product	A formulation is either dispensed by the use of propellant gas as defined in Directive 75/324 (**propellant spray**), or by a spray bottle with a pump dispenser that forces a liquid through a nozzle generating a spray stream or a mist of a liquid (**pump spray**) (SCCS/1539/14).
SSA[1]	Skin Surface Area
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)
SUE (**Serious Undesirable Effects)**	An undesirable effect which results in temporary or permanent functional incapacity, disability, hospitalization, congenital anomalies or an immediate vital risk or death (2009/1223/EC)
T25	Animal dose-descriptor; chronic dose rate that will give 25% of the animal's tumours at a specific tissue site after correction for spontaneous incidence (Dybing *et al.,* 1997)
TER	Transcutaneous Electrical Resistance
TIF	Technical Information File
Toxicodynamics	Cover the process of interaction of chemical substances with target sites and the subsequent reactions leading to adverse effects (ECB, 2003)
Toxicokinetics	Describe the time-dependent fate of a substance within the body and include absorption, distribution, biotransformation and/or excretion (ADME) (ECB, 2003)
TSE	Transmissible Spongiform Encephalopathy
TTC	Threshold of Toxicological Concern
Undesirable effect	An adverse reaction for human health attributable to the normal or reasonably foreseeable use of a cosmetic product (2009/1223/EC)

[1] Used in the calculation of the Systemic Exposure Dose (see Section 3-12.2).

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 EURL-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
XME	Xenobiotic substances Metabolising Enzyme