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Final version

Scientific Committee on Consumer Safety

SCCS

THE SCCS NOTES OF GUIDANCE FOR THE TESTING OF COSMETIC INGREDIENTS AND THEIR SAFETY EVALUATION 10TH REVISION

The SCCS adopted this guidance document
at its plenary meeting on 24 – 25 October 2018

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Nam et ipsa scientia potestas est
For knowledge itself is power

Francis Bacon (1561 - 1626) Essays

The "Notes of Guidance for the 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 indirectly 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 in recent years was the full implementation of the cosmetic legislation, Regulation (EC) No 1223/2009, meaning that the animal testing and marketing bans fully apply from 2013 onwards: no *in vivo* testing of finished products after March 2004; no *in vivo* testing for local toxicity after March 2009 and no *in vivo* testing for repeated dose toxicity (including sensitisation) toxicokinetics and developmental toxicity from March 2013 onwards for the purpose of cosmetics. For this reason, the SCCS has closely followed the progress made with regard to the development and validation of alternative methods, with emphasis on replacement methodology.

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 2015 (SCCS/1564/15). 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. Focus is on exposure and the application of alternative methods, more specifically on non-animal methods.

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" 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 the scientific committee SCHEER and Cosmetics Europe is gratefully acknowledged.

The Chairperson

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SCCS members listed below are acknowledged for their valuable contribution to the finalisation of this guidance document.

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ABOUT THE SCIENTIFIC COMMITTEES

Two independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
These Committees are: the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) and they are made up of scientists appointed in their personal capacity.

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.).

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

Applicants are invited to visit the SCCS website:
https://ec.europa.eu/health/scientific_committees/consumer_safety/opinions_en
where Applicants will find a checklist
for submitting a safety dossier of a cosmetic ingredient.

Applicants are invited to visit the following website for further legislative information:

https://ec.europa.eu/growth/sectors/cosmetics/legislation_en

MAIN CHANGES IN 10TH REVISION OF THE SCCS NOTES OF GUIDANCE (NOG)

Structural changes

- The general structure of the Notes of Guidance has been changed to give priority to non-animal methods, followed by older *in vivo* strategies.
- Information on Regulation 1223/2009/EC and guidance related to finished cosmetic products (Product Information File, PIF) is now included in the Annexes.
- A (revised) checklist for submitting a safety dossier to the SCCS has been added.
- An update of the data needed for safety evaluation has been included and information for re-submitting is also given in case of a negative opinion.
- References and abbreviations lists have been updated.
- The exposure chapter has been updated with subdivisions for external and internal exposure – tiered approach and aggregate exposure are discussed.

Changes in content

- An update on non-animal toxicological studies for cosmetic ingredients has been included.
- Weight of Evidence (WoE) and use of a 'toolbox' (genotoxicity/mutagenicity) are discussed.
- An update of criteria for multi-constituent natural ingredients and chemical identity has been included.
- The Threshold of Toxicological Concern concept – TTC - remains the same, *i.e.* new suggested values in publication have not yet been evaluated.
- A literature overview of consumer exposure data has been added in Appendix 7.

1. INTRODUCTION

Since July 2013, Regulation (EC) No 1223/2009 applies for cosmetic products. Their safety-in-use is, as was also the case for Directive 76/768/EEC, established by controlling the safety of the ingredients.

For those ingredients for which some concern exists with respect to human health (*e.g.* colourants, preservatives, UV-filters, hair dyes), safety evaluation is done at the Commission level by the Scientific Committee on Consumer Safety (SCCS). These substances are addressed in the Annexes of Regulation (EC) No 1223/2009.

For the safety evaluation of cosmetic ingredients, all available scientific data are considered, taking into account the testing and marketing bans in force under Regulation (EC) No 1223/2009. This includes the physical and chemical properties of the compounds under investigation, *in silico* data such as results obtained from (Q)SAR {(Quantitative) Structure Activity Relationship} modelling, chemical categories, grouping, read-across, Physiologically-Based PharmacoKinetics (PBPK) / ToxicoKinetics (PBTK) modelling, *in vitro* and *ex vivo* experimental results and data obtained from animal studies (*in vivo*) that have been carried out for the purpose of cosmetics before the testing and marketing bans.
The animal testing ban on finished cosmetic products applies since 11 September 2004; the testing ban on ingredients or combination of ingredients applies since 11 March 2009. The marketing ban applies since 11 March 2009 for all human health effects with the exception of repeated-dose toxicity, reproductive toxicity, and toxicokinetics. For these specific health effects, the marketing ban applies since 11 March 2013, irrespective of the availability of alternative non-animal methods.
In addition, clinical data, epidemiological studies, information derived from accidents, data from Post-Marketing Surveillance (PMS) or other human data are also taken into consideration.

In the present update, the state-of-the-art with respect to the validated methods of the 3Rs (Refinement, Reduction and Replacement) strategy of Russell *et al.* (1959), is incorporated with emphasis on New Approach Methodologies (NAMs). In view of the testing and marketing bans in the cosmetic regulation, the SCCS gives special attention to those alternative methods that are suitable for the safety testing of cosmetic substances.

Although the "Notes of Guidance" are concerned with the testing and safety evaluation of the cosmetic substances listed in the Annexes of Regulation (EC) No 1223/2009 and those for which safety concerns have been expressed, they could be also of interest for all substances intended to be incorporated in a cosmetic product. Even though the "Notes of Guidance" have not been written for the latter purpose, they can indeed be of practical use in making a Product Information File (PIF) for a finished cosmetic product as currently required by Regulation (EC) No 1223/2009.

The "Notes of Guidance" should not be seen as a prescriptive procedure, but rather as an approach that may need to be adapted on a **case-by-case** basis when evaluating the safety of the Annex substances. However, when major deviations from standardised protocols/procedures in the safety evaluation process have been adopted, it is essential that Applicants provide scientific justification.

The "Notes of Guidance" will be revised as scientifically required on the basis of scientific advances in toxicology and validated alternative methods or legislative changes.

2. THE SCIENTIFIC COMMITTEE ON CONSUMER SAFETY, SCCS

2-1 HISTORICAL BACKGROUND

The SCCS with its current mandate and composition was established in 2016 and will be active until March 2021.
For more information, see **Appendix 1**.

2-2 MANDATE

The SCCS is an advisory body that provides the Commission with scientific advice and safety evaluations for Annex substances and compounds for which some concern for human health exists. Its consultation for this task is compulsory.
For more information, see **Appendix 1.**

2-3 RULES OF PROCEDURE

The SCCS works with 3 working groups, dealing with:

- cosmetic ingredients
- methodology
- nanomaterials.

Safety evaluations and advice are taken up in opinions, which are adopted during a plenary meeting (or by written procedure). A commenting period is foreseen for draft opinions before they are finalised and published.
For more information, see **Appendix 1.**

2-4 OPINIONS

Opinions are published on the SCCS website:
https://ec.europa.eu/health/scientific_committees/consumer_safety/opinions_en.
For more information, see **Appendix 1.**

2-4.1 THE "NOTES OF GUIDANCE"

One of the responsibilities of the 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 the Testing of Cosmetic Ingredients and Their Safety Evaluation' (NoG) that are regularly revised and updated in order to incorporate new knowledge and scientific and regulatory advances. Therefore, dossiers submitted to the SCCS should be in accordance with the latest published version of the NoG. The 9th Revision SCCS/1564/15 of 25 April 2016 is now replaced by this 10th Revision SCCS/1602/18.

As cosmetic ingredients are chemical substances, the NoG include the toxicological test procedures reported in Commission Regulation (EC) No 440/2008. The latter describes 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. Whereas the testing procedures for chemical substances take the 3Rs-principle into consideration, animal experiments for cosmetic purposes are excluded in the EU.

For the safety evaluation of cosmetic ingredients only validated non-animal methods may be applied. Furthermore, testing procedures in accordance with the Organisation for Economic Co-operation and Development (OECD) Guidelines, and, on a case-by-case basis, well documented scientifically-justified alternative methods that may not have been officially validated yet are also carefully considered. Data obtained from animal experimentation for the purpose of cosmetics or other consumer products legislation and generated before the established cosmetic deadlines of the testing and marketing bans (see 1. Introduction) still may be used in the safety evaluation of cosmetics and their ingredients.

For the SCCS' safety evaluation, the systemic doses obtained (mostly) after oral administration are used. For local toxicity endpoints normally only hazard identification is carried out. Safety evaluation is done for intact skin.

2-4.2 SCCS COSMETIC INGREDIENT DOSSIERS

Regulation (EC) No 1223/2009 requires Annexed cosmetic substances to be notified, safety assessed and adequately labelled before being allowed on the EU market. These annexes lay down clear limitations and requirements for the cosmetic substances concerned. The safety assessment of the cosmetic ingredients in the EU is overseen by the SCCS. The evaluations carried out by the SCCS are based on safety dossiers submitted by Applicants (individual company/associations, Competent Authorities).

In view of the animal testing and marketing bans of cosmetic ingredients/products, two main routes to developing safety dossiers are possible:

- In case a new ingredient is specifically developed for use in a cosmetic product, testing needs to be in compliance with the restrictions on animal testing placed under Regulation (EC) No 1223/2009 and safety data need to be derived from non-animal alternative methods.
- Where an ingredient has pre-existing safety data derived from animal tests (*e.g.* an existing cosmetic ingredient) that have been carried out before the regulatory deadlines, it can still be used. Animal test data relating to chemical substances developed for uses other than cosmetics (*e.g.* food, medicines, biocides, etc.) can also be used for supporting safety assessment of an ingredient intended to be used in a cosmetic product.

More details are given in section 1. Introduction.

In case of a negative or inconclusive opinion by the SCCS, resubmission of a dossier is only possible when the Applicant provides sufficient (new) evidence to address the concerns raised.

2-4.3 SPECIFIC ISSUES TAKEN UP IN NOG

In addition to the regular revision of the NoG and the study of toxicological dossiers of cosmetic substances for inclusion in one of the Annexes of Regulation (EC) No 1223/2009, in the following sections some specific issues are addressed. Examples include (non-exhaustive list):

- Alternative methods in the safety assessment of cosmetic ingredients
- Cosmetic ingredients of animal / human origin
- CMR (Carcinogenic, Mutagenic, toxic to Reproduction) issues
- Safety assessment of hair dyes and colourants
- The inventory of cosmetic ingredients (INCI-list)
- Safety of infants and children
- Fragrance allergy in consumers
- Safety assessment of nanomaterials
- Risk and health effects: miscellaneous

3. SAFETY EVALUATION OF COSMETIC INGREDIENTS

3-1 SAFETY EVALUATION OF COSMETIC INGREDIENTS AS APPLIED BY THE SCCS

- **The safety of cosmetic products is based on the safety of the ingredients**

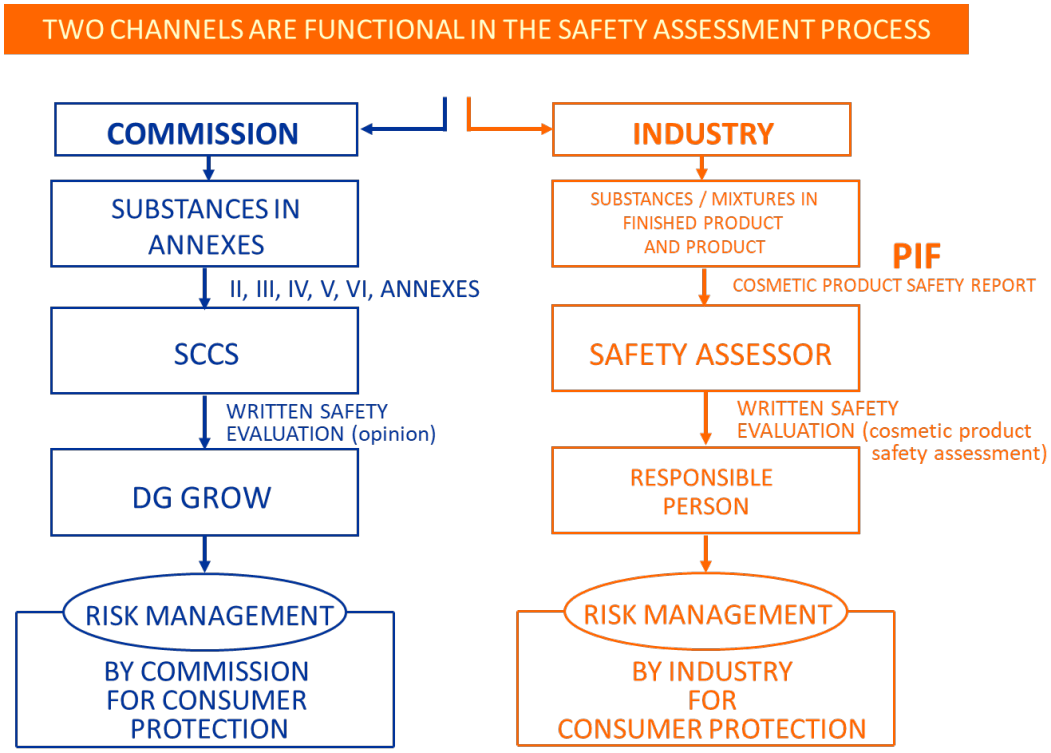
The rationale behind the safety of the cosmetic product being based on the safety of its ingredients comes from the fact that many thousands of different cosmetic products on the EU market are all derived from a limited number of substances. Hence, toxicity testing has been concentrated on ingredients, and particularly on those that are intended to react with biological moieties and therefore are of potential concern for human health. This is also the basis for the lists of authorised and banned and restricted substances (**Table 1**).

Annex II	List of prohibited substances
Annex III	List of restricted substances
Annex IV	List of allowed colourants
Annex V	List of allowed preservatives
Annex VI	List of allowed UV-filters

Table 1: Annexes to Regulation (EC) No 1223/2009

- **For the safety evaluation of cosmetic ingredients two channels are functional**

The safety of the Annex substances is evaluated by the SCCS; the safety of cosmetic products with all their ingredients is evaluated by the industry placing them on the EU market. Thus, the Annex substances fall under the responsibility of the SCCS (left part of **Figure 1**). All the ingredients in cosmetic products are the responsibility of the "Responsible Person, RP", as defined by Regulation (EC) No 1223/2009, through the safety assessor (right part of **Figure 1**).



PIF: product information file.

Figure 1: Human health safety evaluation of cosmetic ingredients in the EU.

- **This guidance, in principle, equally applies to the safety evaluations carried out by the SCCS as by the safety assessors of the cosmetic industry.**

Safety evaluation is generally performed taking into account the data provided by the industry or in some cases by Members States authorities. The SCCS also has the opportunity to add relevant data from the open literature or other relevant sources.

In general, the safety evaluation of cosmetic ingredients by the SCCS is based upon the principles and practice of the risk assessment process universally applied for chemical substances with the challenge that only validated replacement methods (or demonstrated to be scientifically valid) should be used when testing for the purposes of the EU cosmetic legislation.

A typical safety evaluation procedure comprises the following elements:

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, *ex vivo, in vitro* tests, *in silico* methods and read-across, clinical studies, case reports, epidemiological studies and data from Post-Marketing Surveillance (PMS). Intrinsic physical and chemical properties of the substance under consideration are also taken into account.

2) Exposure assessment
   Human exposure is calculated based on the declared functions and uses of a substance as cosmetic ingredient, the amount present in the respective cosmetic product categories and their frequency of use.

   The single product exposure describes the exposure to a cosmetic ingredient in one product category *via* one route.

   The aggregate exposure, in the context of the NoG, is the sum of all relevant single product exposures, so that it describes the exposure from all product categories in which the cosmetic ingredient is used and all relevant exposure routes

   Where necessary, exposure of vulnerable consumer groups could be assessed separately (*e.g.* children, pregnant woman, etc.)

   Generally, only exposures from the use of a substance as cosmetic ingredient are considered, with the exception of CMR compounds, for which non-cosmetic uses should also be taken into account (see section 3-6.6 and **Appendix 5**).

3) Dose-response assessment
   For the relationship between the exposure and the toxic response, a Point of Departure (POD) is determined. The POD is defined as the dose-response point that marks the beginning of a low-dose extrapolation (for threshold and non-threshold compounds). In most Opinions a No Observed Adverse Effect Level (NOAEL) has been used as POD.

   The SCCS considers that, where usable *in vivo* data are available, the preferred method for both threshold and non-threshold cosmetic ingredients is to express the dose metric as Benchmark Dose (BMD). Both the European Food Safety Agency (EFSA) and the World Health Organization (WHO) also recommend that the BMD approach for deriving the POD should be used as a starting point for human health risk assessment.

   The BMD approach has a number of advantages over using NOAEL or the dose-descriptor T25 (dose giving a 25% incidence of cancer in an appropriately designed animal experiment) or TD50 (median toxic dose):
   - it makes complete use of the available dose - response data
   - it takes into account the shape of the dose - response curve
   - it is less dependent on dose spacing
   - it enables quantification of the uncertainties in the dose - response data using statistical methodology (EFSA, 2016).

For compounds with a threshold, the POD can be a NOAEL, a Lowest Observed Adverse Effect Level (LOAEL), or a BMD Lower limit (BMDL) (for details of the NOAEL and BMD approach, see Sections 3-4.8, 3-5.1)

4) Risk characterisation

In risk characterisation, the focus in the NoG is on systemic effects. In the case of a threshold effect, the Margin of Safety (MoS) is mostly calculated from oral toxicity studies, unless robust dermal toxicity data are available1. In the case of an oral toxicity study, the following equation is used:

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

The POD_{sys} is a dose descriptor for the systemic exposure to a substance and is calculated from the oral POD by use of the proportion of the substance systemically absorbed. SED represents the Systemic Exposure Dose (see also Section 3-3.5.4). In this equation, POD_{sys} is BMDL or, alternatively, NOAEL or LOAEL, where BMDL cannot be calculated.

For non-threshold effects (*e.g.* a non-threshold carcinogenic effect), the lifetime risk is often based on the BMD10 (benchmark dose response for a 10% response). The risk assessment of carcinogens is described in Section 3-4.11.

Risk characterisation is followed by risk management and risk communication, which are not in the remit of the SCCS, but of the European Commission (**Figure 1**) and, if it is in regard to a finished cosmetic product and its ingredients, the RP.

Besides the normal procedure when the industry or Member States or their representatives submit 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 and need to be readable and searchable.

Other common formats that allow copy/paste actions are accepted. Scanned documents that are not readable/ searchable are not accepted.

It is beyond the scope of the NoG to discuss the whole process of risk assessment. Numerous review articles and textbooks exist on this topic. The aim is to highlight some key aspects to explain why certain data and test results should be provided in the dossiers on the cosmetic substances presented to the SCCS for evaluation.

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

The framework of a typical dossier is given in **Appendix 3.**

[1] For the case that a dermal repeated dose toxicity study is used, see Section 3-4.8 and 3-5.1

3-2 CHEMICAL AND PHYSICAL SPECIFICATIONS OF COSMETIC INGREDIENTS

Physical and chemical properties of substances are considered as crucial information, since they may indicate potential risks. For example, a small Molecular Weight (MW) hydrophobic compound is more likely to penetrate through the skin than a high MW hydrophilic compound. Physical and chemical properties also identify physical hazards of the substance (*e.g.* corrosiveness as indicated by pH of aqueous solution, volatility, 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) MW;
4) Characterisation and purity of the chemical, including isomer composition whenever relevant for safety assessment;
5) Characterisation of the impurities or accompanying contaminants;
6) Solubility;
7) Partition coefficient (Log P_{ow});
8) Vapour pressure (volatile liquids);
9) Homogeneity and stability;
10) Further physical and chemical properties if relevant for safety evaluation.

For nanomaterials, special requirements for provision of physicochemical data apply (see Section 3-6.7). 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 for the test chemical used to generate the data as submitted in the dossier to the SCCS.

Preference is clearly given to measured parameters of relevant batches on the market over calculated values (*e.g.* log P_{ow}) or literature data (where often batches are tested that differ from the batches used in toxicological tests and therefore may have different composition / impurity profiles).

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

3-2.1 CHEMICAL IDENTITY

The precise identity and chemical nature of the substance under consideration and its structural formula must be given. The Chemical Abstracts Service (CAS) number of the chemical, the International Nomenclature of Cosmetic Ingredients (INCI) name or Common Ingredient Nomenclature (CIN) name and the EC number (see **Appendix 2** 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 complex mixture (*e.g.* an extract), complete information should be provided on the origin of the source materials (*e.g.* part of a plant), extraction method and any additional processes and/or purification steps used (see Section 3-6.1) to establish a standardised material as representative of the extract present in commercial products.

In case of a mixture, all components must be described in terms of qualitative and quantitative formulae. These could be: main components, preservatives, antioxidants,

chelators, buffering agents, solvents, other additives, impurities and/or additional external contamination.

When a cosmetic ingredient and its derivatives (salt, ester, …) are submitted for evaluation, this must be clearly specified in the dossier, because the chemical form can determine the safety evaluation. The physical and chemical properties of all specific chemical forms must be provided, and the same specific substances must be used in the toxicological studies performed for the safety evaluation. Any deviations must be justified.

3-2.2 PHYSICAL FORM

A description of the physical form should be given: powder, paste, gel, liquid. For nanoparticles, further information as specified in Section 3-6.7 should be given, including the particle size and its distribution.

For polymer ingredients, the molecular weight distribution should be provided.

3-2.3 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.

3-2.4 IDENTIFICATION AND PURITY OF THE CHEMICAL AND ISOMER COMPOSITION

The degree of purity must be clearly indicated. 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 **(Appendix 6)** 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 High Performance Liquid Chromatography (HPLC) recovery of the test material is clearly documented,
3) the UV detection of the active substance is performed at λ_{max} , in an appropriate mobile phase, and
4) peak purity of the active substance is clearly documented.

The experimental conditions of the techniques used for the chemical characterisation (Ultra Violet (UV), Infra Red (IR) and Nuclear Magnetic Resonance (NMR) spectroscopy, Mass Spectrometry (MS), chromatographic techniques *e.g.* Gass Chromatography (GC), 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.

3-2.5 CHARACTERISATION OF THE IMPURITIES OR ACCOMPANYING CONTAMINANTS

In addition to the purity of the substance under consideration, identity in terms of chemical nature and concentration of impurities that may be present must also be stated. Impurities should be characterised and quantified by an appropriate analytical method, *e.g.* by HPLC-PDA (Photometric Diode Array), LC-MS/GC-MS, NMR spectroscopy etc., using reference standards with documented purity, where appropriate. Validated analytical procedures should be used for impurity testing. 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, it must be ensured 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 any purification measures, is important. A change in these processes will need careful re-evaluation of the impurities, even if the level of purities remains the same.

3-2.6 RELEVANT PHYSICOCHEMICAL SPECIFICATIONS

A typical physicochemical dataset consists of:

- Physical state (solid, liquid, gas)
- Organoleptic properties (colour, odour, taste if relevant)
- Solubility (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: morphological form (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, the UV spectrum is indispensable.
- For nanomaterials and nanoparticles special requirements apply (see Section 3-6.7).

3-2.7 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 media or other solvents. These should be clearly stated.

Where the solubility of the active substance in water is low (according to EU Method A.6), a highly sensitive and selective analytical technique (such as 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.

Similarly, solubility of substances that are poorly soluble in various solvents should be measured by highly sensitive and selective analytical technique (such as LC/MS).

In cases of low solubility of the active substance in reverse phase HPLC mobile phases, sensitive detection systems, such as MS, should be applied, or other normal phase chromatography should be used.

The solubility of the active substance in the solvent systems used in various studies should also be clearly presented.

3-2.8 PARTITION COEFFICIENT (LOG P_{ow})

The n-octanol/ water partition coefficient (EC A.8) should be given, along with the pH and temperature conditions.

In the case of a calculated value, the method used for estimation should be specified.
LogP_{ow} values often depend on the pH, especially for ionisable molecules, zwitterions, etc. Therefore, a single calculated value of Log P_{ow}, without any reference to the respective pH, cannot be correlated to the physiological conditions and the pH conditions of the dermal absorption studies.

3-2.9 HOMOGENEITY AND STABILITY

Homogeneity data of the test solutions with respect to the content of the test substance, under experimental conditions, should be provided.

Data on the stability of the test substance under the experimental conditions of the reported studies and under conditions of use should be provided. Validated analytical procedures should be used to determine stability of the test substance. In addition, the stability of the test substance relating to its thermal stability and, if applicable, sensitivity to moisture or oxygen under storage conditions and in typical cosmetic formulations should also be provided. Any degradation products should be chemically characterised. In this regard, it is important that the storage conditions and the lengths of studies chosen should be sufficient to cover the storage, shipment, and subsequent use. The stability studies should also be conducted on the test substance packaged in a container, which is the same as the container intended for storage and distribution for marketing.

3-3 EXPOSURE ASSESSMENT

3-3.1 FUNCTIONS AND USES OF COSMETIC INGREDIENTS

For substances that are evaluated as cosmetic ingredients, the concentration, function and way of achieving that function in marketed cosmetic products should be reported. In particular, it should be explicitly mentioned whether substances are meant to be included in sprays or aerosols since consumer exposure *via* inhalation is then probable and needs to be taken into consideration in the overall risk assessment.

In addition, other uses of the substance (*e.g.* in consumer products, industrial products) and, wherever possible, the concentrations involved in such uses should be described.

3-3.2 IDENTIFICATION OF RELEVANT EXPOSURE SCENARIOS

In order to assess exposure of the end users, relevant exposure scenarios have to be identified that comprise all the important functions and uses of a cosmetic ingredient (see Section 3-3.1). These scenarios need to describe "reasonably foreseeable exposure conditions" (Cosmetics Regulation (EC) No 1223/2009, Article 16 f), under which these the cosmetic product should be safe.

The following parameters describe an exposure scenario. However, the list is not exhaustive, and further parameters may need to be taken into account. Note that all routes of exposure (dermal, oral and inhalation) should be considered in view of the intended use of the product.

- cosmetic product type (s) in which the ingredient may be used
- method of application as detailed as possible, *e.g.* rubbed-on, sprayed, applied and washed off, etc.; considerations whether the product is a rinse-off or leave-on product and which retention factor should be applied
- concentration of the ingredient in the marketed cosmetic product
- quantity of the product used at each application
- frequency of use
- total area of skin contact
- duration of exposure
- target consumer groups (*e.g.* children, people with sensitive, damaged or compromised skin) where specifically required
- application on skin areas exposed to sunlight
- location of use (indoors/outdoors) and ventilation

3-3.3 IDENTIFICATION OF THE TARGETED DOSE FOR SAFETY EVALUATION

The hazard identification can either point to systemic effects that require comparison to a SED or local effects, like skin/eye irritation, skin sensitisation, sun-induced skin reactions or effects on the lungs, which mostly are dependent on the amount of substance acting on the surface tissues of the respective body part and require comparison to a Local External Dose (LED).

In the exposure assessment, first the LEDs are calculated that are expected at the specific body entrances and available for uptake. The most important body entrances for substances in cosmetics are the skin, the inhalatory tract and the mouth. These correspond to the uptake routes for internal exposure (dermal route, inhalation route and oral ingestion). For selected products other entrances are possible *e.g. via* the eyes (*e.g.* eye makeup), or *via* genital regions (*e.g.* intimate spray, intimate creams).
As an example, the LED in the lung (the amount of compound per g of lung tissue) can be compared to a "local" NOAEL, and a local MoS can be calculated for effects on the lungs.

The external exposure can further be used to calculate internal (or systemic) exposure which corresponds to an internal dose (see Section 3-3.5.4). For the calculation of the SED, absorption (or uptake) specific to the respective exposure route has to be taken into account.

For risk assessment, the Margin Of Safety (MoS) (see Section 3-5.1) is based on the internal dose, *i.e.* the SED.

3-3.4 EXTERNAL EXPOSURE

3-3.4.1 EXPOSURE MODELS AND TIERED APPROACH

Exposure is calculated based on exposure scenarios by using appropriate exposure models. Generally, external exposure is calculated by multiplying the concentration/fraction of a substance in a source with the amount of the source that is applied on, or reaches, a specified site. To save time and resources, a **tiered approach** is normally followed that first investigates exposure based on generic exposure scenarios with conservative point values as model parameters (screening level).

Where necessary, these conservative exposure estimates are refined in a higher tier by using probabilistic approaches or other means of refinement (Meek *et al,* 2011).
For the safety evaluation of cosmetics, such a screening level approach is the calculation of aggregate exposure according to the NoG (see **Tables 2A** and **2B**). The parameter values presented there can be used as the basis for a deterministic first-tier assessment. If a refinement is necessary, a probabilistic approach can be followed by the use of appropriate models and/or tools. However, this needs to be clearly justified. For regulatory purposes, the probabilistic approach needs to be conservative but realistic and transparent.

In particular, for probabilistic assessments the SCCS recommends the following:

- Habits and practices in a population regarding the use of product categories may be treated probabilistically, under the assumption that they will not change rapidly over time.
- The target protection goal will be the 95th percentile of the European population. Therefore, for a probabilistic assessment the relevant SED for deriving the MoS will be the 95th percentile of the probabilistically assessed population exposure.
- Ingredient concentrations in product categories should normally cover the worst case, *i.e.* for ingredients with restrictions on concentrations and applicability domains (Annex III of the EU Cosmetic Regulation), also in the probabilistic assessment the maximal allowed concentrations should be used, and for other ingredients the maximal concentrations that are realistically foreseeable in a specific product category. This is because product formulations may be highly variable over time, so that an assessment of ingredient concentrations at a specific point in time may not cover the use of the ingredient in the future.
- For reasons of transparency, the model equations and the input parameters need to be provided together with the exposure estimates, so that the exposure calculation is reproducible. If this is not possible, because a specific tool has been used, the original input file containing used distributions and all settings, and the original output file need to be provided by the Applicant. The output file needs to contain the date of the assessment, the relevant model settings and parameters for this assessment and the associated results, ideally not only in tabular form by giving relevant percentiles of the exposure distribution, but also by graphical visualisation.

3-3.4.1.1 DERMAL EXPOSURE MODELS

For cosmetics, the dermal route is often the most important one.

Apart from the general approach, the calculation of dermal exposure needs to take into account that only a fraction of the product is retained on the skin. Therefore, a retention factor F_{ret} is used that represents the fraction available for uptake. For leave-on cosmetics (*e.g.* creams, body lotion, etc.) mostly a fraction of 1 (100%) is used, while for rinse-off cosmetics (*e.g.* shower gel, shampoo, etc.) a smaller fraction is used that depends on the respective product. In **Table 2** retention factors are listed that are applied by the SCCS.

External dermal exposure (E_{dermal}) per day for a substance from a certain product category $_x$ can be calculated according to:

$$E_{dermal\,x} = C_x \times q_x \times F_{ret\,x} \qquad (1)$$

$E_{dermal\,x}$ (mg/day):	external exposure available for dermal uptake from product category $_x$
$_x$:	product category
C_x (mg/g):	concentration/ fraction of a substance in a product category$_x$
q_x (g/day):	amount of product category that is applied/received per day
$F_{ret\,x}$:	retention factor specific to product category $_x$

The daily amount (q_x) and retention factor ($F_{ret\,x}$) are specific to the product category under consideration, and do not depend on the substance. When multiplied, they yield the daily effective amount per product category, Eproduct = q_x X $F_{ret\,x}$, which is listed in **Table 2** for the most important product categories. Multiplied with the concentration or fraction of a substance in a product, they yield the external dermal exposure to a substance per product category $E_{dermal\,x,}$ as shown in equation (1).

This external exposure can be used to calculate the SED by multiplying with the chemical- and route-specific uptake rate and normalisation by the bodyweight (see chapter 3-3.5.4).
In cases where the amount per day q_x is not given or if more detailed probabilistic assessments should be performed, the amount per day can be calculated from the frequency of application (**Table 3**) and the amount per application. In **Appendix 7** (**Table A.7**) a literature review can be found listing studies which provide detailed external exposure values to different cosmetic products. These are given for specific countries.

Further, the external daily exposure per product category can be used to derive a LED. Normally, local dermal effects depend on the surface load, so that the total dermal exposure is normalised by the surface area of application:

$$LED_x = E_{dermal\,x} / SSA \qquad (2)$$

LED_x (mg/day/cm^2): local external dose from a product category x

SSA (cm^2): skin surface area

$E_{dermal\,x}$ (mg/day): external exposure available for dermal uptake from product category x

3-3.4.1.2 ORAL EXPOSURE MODELS

The same principles as described for dermal exposure can be applied for oral exposure. Ingestion can be calculated according to equation (2) by applying adequate retention factors. Such oral retention factors are needed to take into account that only a fraction of the orally applied products will be ingested. Since orally applied cosmetics such as toothpaste, mouthwash or lipstick are normally not intended to be ingested, such retention factors will normally be low.

3-3.4.1.3 INHALATION EXPOSURE MODELS

Cosmetic substances can be inhaled in the form of powder, vapor, aerosolised droplets or aerosolised particles.

For powders, the principles are very similar to spray products. Inhalation exposure to cosmetic powders during intended use usually is limited and the safety of airnborne particles depends in particular on the aerodynamic diameter of the particles. In the safety evaluation of powders, the robustness of the exposure data plays a major role (Steiling *et al*., 2018).

Vapors result from the transfer of volatile substances into the air after dermal or spray application of products or due to evaporation of substances. Non-volatile substances can be transferred into the air mechanically by spraying, where they are initially present in the form of small droplets or particles.
External exposure to vapor can be calculated directly based on the concentration of the substance in the air, whereas for aerosolised particles and droplets, the deposition efficiency in the respiratory tract has to be considered.

This deposition is size-dependent. The size of the droplets after spraying is influenced by the actual formulation (surface tension) and by the vapour pressure of 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.

Generally, there are two types of spray applications: propellant driven aerosol sprays and pump sprays. According to Bremmer *et al.* (Bremmer *et al.,* 2006a; Bremmer *et al.,* 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 (Quadros and Marr, 2011) have shown that pump sprays can even 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. For more detailed considerations, see Guidance on the Safety Assessment of Nanomaterials in Cosmetics (SCCS/1484/12, under revision).

A sprayed formulation generally consists of droplets of different sizes and/or particles which changes its composition with time (*e.g.* by aggregation of particles 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 as inhalable. This is different from laboratory animals, *i.e.* rodents that inhale and exhale through their nostrils, where only particles with a MMAD < 1 to 5 µm are capable of reaching the lung.

For humans, 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). Relative to total airborne particles, the particle size having 50% penetration for the thoracic and respirable fractions are 10 µm and 4 µm, respectively. Estimates for adults and children during typical activities with both nasal and oral inhalation have been determined by Brown *et al.* (Brown *et al,* 2013).

Particle deposition in the lung depends not only on particle size, but also on density, and hygroscopicity (ability of a substance to attract and hold water molecules from the surrounding environment) and is influenced by the local anatomy and airflow (Braakhuis *et al.,* 2014).

After mucociliary clearance, further intake of insoluble particles or their components *via* the oral route may occur in humans.

The level of exposure can be directly measured under standard exposure conditions, or by using mathematical models.

When measuring exposure, it is important to do it during the relevant exposure period after spraying, under relevant conditions (Carthew *et al.*, 2002; Rothe *et al*., 2011). Default equations can be used as a conservative, worst case approach, and as a first estimate (ECHA, 2012b). For a more realistic assessment, one of the tools that can be considered to assess exposure to solvents or exposure to aerosols generated after the use of spray applications is the ConsExpo model (www.consexpo.nl). This tool comprises two modules for inhalation: 1) exposure to vapour and 2) exposure to sprays.

The spray module calculates the exposure based on the inhalable fraction of the generated aerosols. For conventional substances, it is assumed that these are homogeneously distributed over the generated aerosols, on a mass basis.

For that reason, in the experiments carried out for the calibration of the model, aerosols with a size <1 µm have not been taken into account. It should be noted that the mass of aerosol droplets <1µm is negligible compared to the aerosols present in the inhalable fraction of 1-20 µm.
Key parameters in the calculation of the inhalation exposure are: room volume, spray duration, ventilation rate, exposure duration and product specific parameters, such as mass generation rate, airborne fraction, aerosol size distribution, and weight fraction of the ingredient. Note that since nanoparticles had not been measured in the calibration data set underlying the model, ConsExpo Spray cannot be used directly for nanoparticles.

Inhalation is not the standard route of exposure for cosmetic exposure. Therefore, the flow chart (see **Figure 2**) can be followed to determine whether assessment of inhalation exposure is necessary for a given cosmetic formulation.

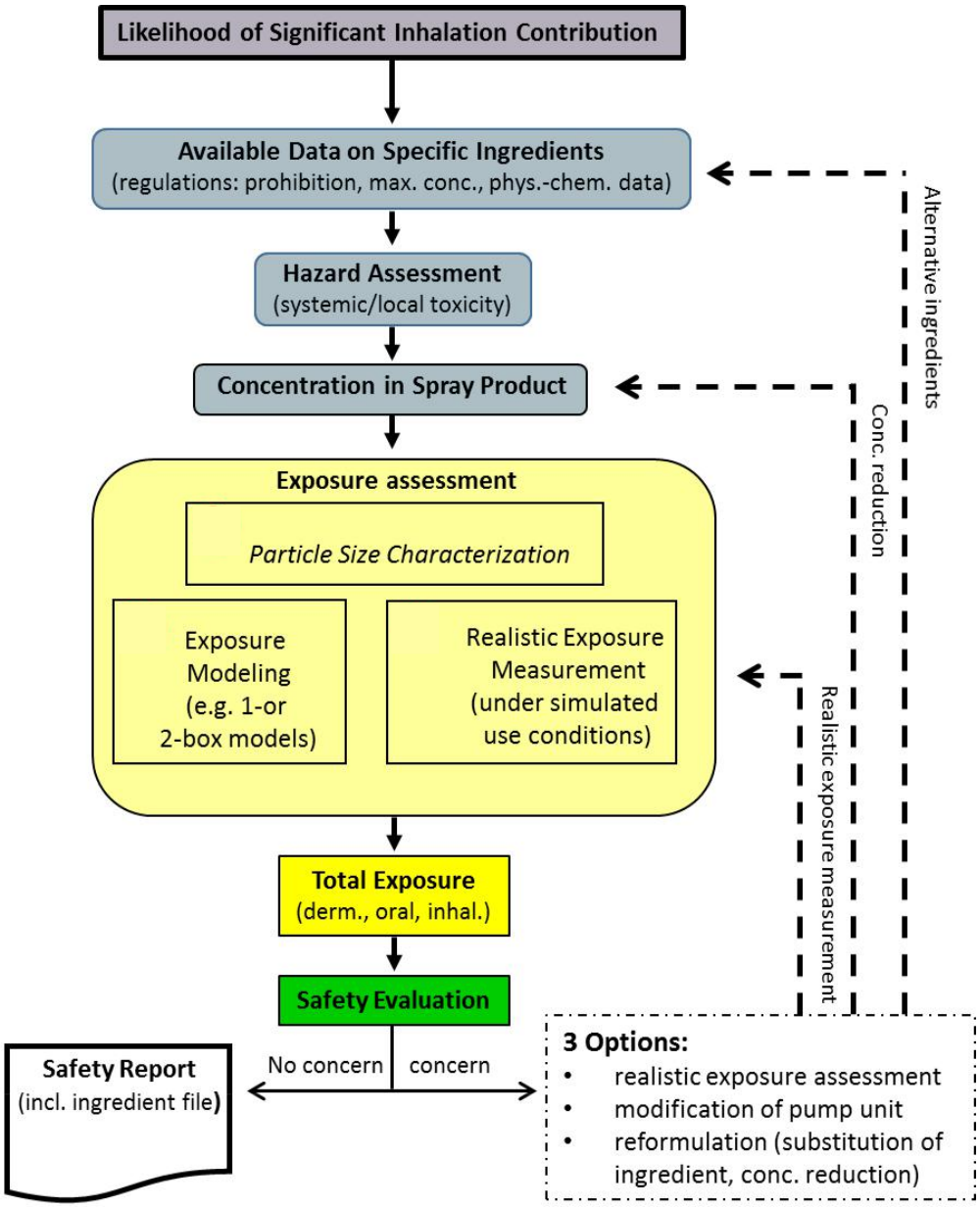


Figure 2: Basic principles for the tiered safety assessment of inhalable cosmetic products and their ingredients. Modified from Steiling *et al.* (2014), grey-related to ingredients; yellow-related to product exposure.

3-3.4.2 MODEL PARAMETERS

For the parameter values, either point values (deterministic assessment) or distributions (probabilistic assessment) can be used. Regardless of the method, the calculation needs to be conservative. In the case of a deterministic assessment this means that higher percentiles should be used for most parameters. In order not to be overly conservative, for some parameters, such as the body weight, a mean or a standard value can be chosen.

3-3.4.2.1 DAILY USE AMOUNTS AND RETENTION FACTORS

Upon request of the SCCS, Cosmetics Europe has provided a large-scale use study for the most important consumer product categories (based on frequency and amount of use in the general population) among consumers in different European Member States. Prediction for the European population was realised by generating daily applied amounts using probabilistic analysis for 11 product categories, i.e body lotion, deodorant, facial moisturiser, shampoo, lipstick, toothpaste, mouthwash, shower gel, liquid foundation, hand cream and hair styling products (Hall *et al.*, 2007; McNamara *et al.,* 2007, Hall *et al.,* 2011). The publications report consumed amounts of cosmetic products per day and per kg bodyweight. They do not differentiate between frequency of application and amount per application based on the assumption that for regularly used products the frequency and amount are inversely correlated.

In **Table 2A** conservative point values for the estimated amount q_x are listed that can be used to assess exposure in a first tier. From the amount distributions generated in the probabilistic assessments (Hall *et al*, 2007, Hall *et al*, 2011), the P90 was chosen for both daily and relative daily amount applied to the skin, respectively. These amounts were multiplied with the respective retention factors F_{ret} (derived in SCCNFP/0321/00) to yield the effective exposure to a product category ($E_{product}$). From the $E_{product}$ derived below the dermal exposure E_{dermal} to a substance can be calculated according to:

$$\mathbf{E_{dermal} = E_{product} \; X \; C_x}$$

where C_x: substance concentration in a product category.

Table 2A: Daily exposure levels for different cosmetic product categories in Europe, calculated by multiplying daily amounts (Hall *et al.* 2007, Hall *et al.* 2011) and F_{ret}.

Product type	*Estimated daily amount applied* q_x *(g/d)*	*Relative daily amount applied*[1] q_x *(mg/kg bw/d)*	*Retention factor*[2] F_{ret}	*Calculated daily exposure* $E_{product}$ *(g/d)*	*Calculated relative daily exposure*[1] $E_{product}$ *(mg/kg bw/d)*
Bathing, showering					
Shower gel	18.67	279.20	0.01	**0.19**	**2.79**
Hair care					
Shampoo	10.46	150.49	0.01	**0.11**	**1.51**
Hair styling products	4.00	57.40	0.10	**0.40**	**5.74**
Skin care					
Body lotion	7.82	123.20	1.00	**7.82**	**123.20**
Face cream	1.54	24.14	1.00	**1.54**	**24.14**
Hand cream	2.16	32.70	1.00	**2.16**	**32.70**
Make-up					
Liquid foundation	0.51	7.90	1.00	**0.51**	**7.90**
Lipstick, lip salve	0.057	0.90	1.00	**0.057**	**0.90**
Deodorant					
Deodorant non-spray	1.50	22.08	1.00	**1.50**	**22.08**
Deodorant spray	0.69	10.00	1.00	**0.69**	**10.00**
Oral hygiene					
Toothpaste (adult)	2.75	43.29	0.05	**0.138**	**2.16**
Mouthwash	21.62	325.40	0.10	**2.16**	**32.54**

1 The specific body weight of the persons involved in the study is used and not the default value of 60 kg.
2 The retention factor (F_{ret}) 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); F_{ret} has no units.

In **Table 2A**, bodyweight (bw) was based on bodyweight distribution was based on bodyweight from several European countries. The represented EU countries were Spain, Great Britain, France, Germany and Denmark in which ''Spain'' = Spain, Italy, Portugal and Greece (southern European countries), Great Britain (''GB'') = UK and Ireland, ''France'' = France, ''Germany'' = Germany, Belgium, Luxembourg, the Netherlands and Austria and ''Denmark'' = Denmark, Finland and Sweden (northern European countries).

In **Table 2B** some estimated daily exposure levels for Europe are present; they were provided earlier by Cosmetics Europe for cosmetic products which were not taken up in the Crème study.

Table 2B: Estimated daily exposure levels in Europe for additional cosmetic product categories, which are not covered by Hall *et al.* 2007, 2011 (SCCNFP/0321/00, Steiling *et al.* 2012, Colipa 16.01.97 BB-97/007).

Product type	*Estimated daily amount applied* q_x *(g/d)*	*Relative daily amount applied* q_x *(mg/kg bw/d)*	*Retention factor*[1] F_{ret}	*Calculated daily exposure* $E_{product}$ *(g/d)*	*Calculated relative daily exposure* $E_{product}$ *(mg/kg bw/d)*
Hair care					
Hair conditioner[2]	3.92	-	0.01	**0.04**	**0.67**
Semi-permanent hair dyes (and lotions)[2]	35 ml (per application)	-	0.01	**Not calculated**[3]	**-**
Oxidative/permanent hair dyes[2]	100 ml (per application)	-	0.01	**Not calculated**[3]	**-**
Make-up					
Make-up remover[2]	5.00	-	0.10	**0.50**	**8.33**
Eye shadow[2]	0.02	-	1.00	**0.02**	**0.33**
Mascara[2]	0.025	-	1.00	**0.025**	**0.42**
Eyeliner[2]	0.005	-	1.00	**0.005**	**0.08**
Deodorant					
Deodorant aerosol spray (ethanol-based)[4]	1.43	20.63	1.00	**1.43**	**20.63**

1 The retention factor (F_{ret}) 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). F_{ret} has no units.
2 Product categories not covered by Hall *et al*., 2005, 2011: existing daily application amounts are divided by the mean human body weight of 60 kg.
3 Daily exposure value not calculated due to the low frequency of exposure.
4 Steiling *et al.,* 2014: 'ethanol-based' are products containing ethanol as principal ingredient.

SCCNFP data (taken up in **Table 2B**) represents the data on consumer exposure arising from normal and extensive use (Colipa 16.01.97 BB-97/007, SCCNFP /0321/00). Data are based on female usage (higher than for males). All categories of cosmetic products were used extensively.

Alternatively, if daily use data are not available, the daily use can be calculated from the frequency of the application event and the amount per event. For calculating the amount per event *e.g.* the surface area of body parts can be helpful. Therefore, in **Table 3** human surface areas (Bremmer *et al*., 2006a; Bremmer *et al*., 2006b) and the frequency of application are provided. For calculating a first tier, the maximum frequency per day should be multiplied by the maximally applied amount. For daily amounts per body weight these amounts can be divided by the mean human body weight of 60 kg.

Table 3: Mean exposed skin surface area per product category (Bremmer *et al.*, 2006a; Bremmer *et al.,* 2006b) and frequency of application per product category

Product type	*Surface area involved* (cm^2)	*Parameters (if specified)*	*Frequency of application*
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 hands	1/day
Hair care			
Shampoo	1440	area hands+ ½ area head	1/day
Hair conditioner	1440	area hands+ ½ area head	0.28/day
Hair styling products	1010	½ area hands+ ½ area head	1.14/day
Semi-permanent hair dyes (and lotions)	580	½ area head	1/week (20min.)
Oxidative/ permanent hair dyes	580	½ area head	1/month (30min.)
Skin care			
Body lotion	15670	area body-area head (female)	2.28/day
Face cream	565	½ area head (female)	2.14/day
(+applied on neck)	320[1]		
(+ applied on back of neck)	80[2]		
Hand cream	860	area hands	2/day
Make-up			
Liquid foundation	565	½ area head (female)	1/day
Make-up remover	565	½ area head (female)	1/day
Eye shadow	24		2/day
Mascara	1.6		2/day
Eyeliner	3.2		2/day
Lipstick, lip salve	4.8[3]		2/day
Deodorant/antiperspirant			
Deodorant spray[4] and non-spray[5]	200	both axillae	2/day
Fragrances			
Eau de toilette spray	200	total body area	1/day
Perfume spray	100	area hands	1/day
Men's cosmetics			
Shaving cream	305	¼ area hand (male)	1/day
Aftershave	305	¼ area hand (male)	1/day
Sun care cosmetics			
Sunscreen lotion/ cream	17500	total body area	2/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.
4 Daily exposure value not calculated due to the low frequency of exposure
5 Steiling *et al.*, 2014: 'ethanol-based' are product categories containing ethanol as principal ingredient.

The SCCS emphasises that it is not the intention to provide parameter values and exposure estimates for **all** cosmetic product categories. Only for the most common categories default values are provided. For all other cosmetic product categories, the individual companies and/or the qualified safety assessors need to make a case-by-case assessment of the daily exposure level and/or the frequency of application. Exposure values, frequency of application and other relevant information for individual cosmetic product categories can be found in **Appendix 7.**

For sunscreen products, an application of **18.0 g/d** is used in the MoS calculation (see also 3-6.4).

3-3.4.2.2 CONCENTRATIONS

As parameter values for concentration, the maximal allowed levels need to be taken into account. If different levels are allowed in different product categories, the category-specific levels should be considered.

3-3.4.2.3 PARAMETERS SPECIFIC FOR INHALATION EXPOSURE

For spray products – propellant or pump sprays the relevant concentration to calculate exposure is not the concentration in the formulation, but the concentration in the spray mist. Finally, according to the explanations in chapter 3-3.4.1.3 (inhalation models), one important parameter is the deposition rate. Deposition rates have, for example, been determined in an International Commission Radiological Protection project (ICRP, 1994).

3-3.4.3 AGGREGATE EXPOSURE

Aggregate exposure is obtained by aggregating (adding up) the exposures to a cosmetic ingredient contained in several single product categories (*e.g.* shampoo, hand cream, etc). It needs to be calculated in the case where several product categories contribute. For the calculation of LEDs the aggregation is specific to the investigated site and if a risk assessment should be conducted for local exposure, the cosmetic ingredient single doses need to be added up for the specific investigated site. In the absence of a valid approach for a quantitative risk assessment of the local effect (which is *e.g.* the case for skin sensitisation), the assessment is hazard-based.

If the external aggregate exposure should serve to calculate SEDs, aggregation needs to take into account all product categories that can be taken up by a specific route. For each route a specific aggregate external exposure needs to be provided. If aggregation over routes is necessary, because different routes (*e.g.* dermal and inhalation route) contribute, aggregation over routes needs to be done on the level of internal exposure.

For aggregate dermal exposure as a first tier, the SCCS recommends to calculate the LEDs and SEDs based on the product category-specific exposures $E_{product}$ given in **Table 4**. For preservatives and other substances that are regulated with the same maximal concentrations in all product categories, the LEDs or SEDs can directly be derived by multiplying the aggregate $E_{product}$ with the maximal allowed concentration (C_x) by skin surface area (SSA in cm^2). For other cosmetic ingredients the respective $E_{product}$ needs to be multiplied with the maximal concentration specific to the product category.

Whenever available, the values in **Table 4** were taken from the $E_{product}$ presented in **Table 2A.** For some product categories probabilistic data were not available and for these categories earlier information provided by Cosmetics Europe was used (**Table 2B**). Note, that the $E_{product}$ for the oral care products in this context is used for calculating the dermal exposure (via mucosa) and not oral exposure. Oral exposure, if applicable, needs to be calculated separately.

Table 4: Product exposures for the deterministic calculation of aggregate exposure for preservatives through cosmetic use.

Type of cosmetic product exposure	***Product category***	***Exposure product ($E_{product}$)*** *(g/d)*	***$E_{product}$ normalized by body weight[1]*** *(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 cleansing products	Body lotion	7.82	123.20
	Face cream	1.54	24.14
	Hand cream	2.16	32.70
	Deodorant 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
	Lipstick	0.06	0.90
	Eye make-up	0.02	0.33
	Mascara	0.025	0.42
	Eyeliner	0.005	0.08
Oral care Products[2]	Toothpaste	0.14	2.16
	Mouthwash	2.16	32.54
TOTAL		**17.4**	**269**

1 The specific body weight of the persons involved in the study is used and not the default value of 60kg
2 Oral care product categories are not corrected and are presumed here to only represent dermal exposure (mucosa)

The consumer may also be exposed to cosmetic substances through inhalation (*e.g.* through spray applications) or oral exposure. These exposure routes are not considered for **Tables 2, 3 and 4** since the inhalation and oral risk is assessed on a case-by-case basis.

3-3.5 INTERNAL EXPOSURE

Internal exposure can either be measured in humans or calculated from external exposure *e.g.* by applying route-specific absorption factors that translate the amount of substance entering the body into the amount that is available in the blood stream and constitutes the dose acting on organ level. In this guidance, this dose is called the SED. There are also other ways to calculate this internal dose, *e.g.* by more realistically describing the toxicokinetics and applying different kinds of PBPK models.

3-3.5.1 TOXICOKINETICS (ADME)

The term "toxicokinetics" is used to describe the time-dependent uptake, distribution and fate of a substance entering the body. This includes Absorption, Distribution, Metabolism and Excretion (ADME). All of these processes need to be known to understand the fate of substances once they enter the body. The testing guidelines for toxicokinetics, including dermal absorption (EC B.36 Toxicokinetics, EC B.44 Skin absorption: *in vivo* method, EC B.45 Skin absorption: *in vitro* method; corresponding with OECD 417, 427, 428, respectively), are designed to elucidate particular aspects of the fate and the potential 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 on the toxic potential. Therefore, in specific cases, *in vivo* or *in vitro* biotransformation studies are required. However, the conduct and use of *in vivo* studies is restricted due to the animal testing ban for cosmetic ingredients in the EU (see Section 2-4.1).

Apart from data on dermal absorption, further toxicokinetic data for cosmetic ingredients are only available under certain circumstances, but their relevance may be high for extrapolating both *in vivo* and *in vitro* animal data to the human situation.

Any route-to-route extrapolation of toxicity 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 3-5.1.

A review of the current status of toxicokinetics in the safety evaluation of cosmetics and their ingredients can be found in several JRC reports (Adler *et al.* 2011, JRC Scientific and Policy Report 2013a, 2014a, b, 2015, 2016, 2017). At present, no validated alternative methods that completely cover the field of ADME exist. Some *in vitro* models could be 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, HepaRG™ cells, and their cultures), but most of the existing models have not been officially validated (Adler *et al.,* 2011; Eskes *et al.,* 2005; JRC Scientific and Policy Report 2013a, 2014a, 2014b, 2015a, 2016, 2017).

In a limited number of cases, human toxicokinetic study results are available to the SCCS for cosmetic ingredients, *e.g.* zinc pyrithione (SCCS/1512/13), cyclopentasiloxane D5 (SCCS/1549/15), phenoxyethanol (SCCS/1575/16) and salicylic acid (SCCS/1602/18). It would be a step forward to include more human toxicokinetic studies in the dossiers of Annex substances provided that a) risk assessment cannot adequately be performed by use of other data/methodologies and b) such human studies are ethically acceptable.

3-3.5.1.1 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 high number of factors influence this process, including the 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, skin integrity, etc.

Recommended procedures and advice with respect to dermal absorption have been given by several international bodies (ECETOC, 1993; US EPA, 1996a; OECD, 200; WHO, 2006; OECD, 2011a). Sometimes, different terminology is used.

a. <u>Guidelines for dermal absorption studies</u>

Skin absorption studies can be performed in principle *in vivo* (OECD 427) or *in vitro* (OECD 428). Detailed guidance on their performance is available (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 OECD 428 guideline with the SCCS "Basic Criteria" (SCCS/1358/10) is considered to be essential for performing appropriate *in vitro* dermal 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 the systemic compartment of the human body under in-use conditions. These amounts can then be taken into consideration to calculate the MoS during risk characterisation.
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. If not available, pig skin is an alternative (Gerstel *et al.* 2016).
4) Skin integrity is of key importance and should be verified. Poor barrier quality may lead to high dermal absorption values. Skin integrity can be measured using a variety of methods (Guth *et al.* 2015, Fasano *et al.* 2002, Lehman *et al.* 2017).
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.

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.

10) 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%.
11) An appropriate number of controls (for *in vitro* studies: diffusion cells) should be used to determine the background level. In cases of high background level and high variability of the background level, it may be necessary to determine it for every single donor in an appropriate number of repetitions.
12) Treatment of non-detects: if measurements are below the Limit Of Detection/ Limit Of Quantification (LOD/LOQ) or below the background level for the calculation of absorption, either the lower bound (zero) or upper bound (LOQ/LOD) can be used. The choice of either upper or lower level needs to ensure that the highest possible absorption value is calculated.

13) Variability / validity / reproducibility of the method should be discussed. The SCCS considers that for a reliable dermal absorption study, 8 skin samples from at least 4 donors should be used. The absorption needs to be calculated for each single diffusion cell and these values should be used to derive the mean absorption. An appropriate number of repetitions should be used for each donor.

14) 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.

15) The technical ability of the performing laboratory and the validity of the method used should be assessed at regular intervals, at least twice per year, by using reference compounds like caffeine or benzoic acid. These data should be included in the study report (OECD, 2004; Van de Sandt *et al.*, 2004).

16) Sample application *in vitro* should mimic human exposure, normally 1-5 mg/cm² for a solid and up to 10 µl/cm² for liquids (OECD 428).

Exceptions may exist, *e.g.,* oxidative hair dyes, where 20 mg/cm² are usually 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 given in percent of the test dose is used to calculate SED, they may result in an underestimation of systemic exposure.

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 may be specifically added to a cosmetic formulation to facilitate the dermal absorption of certain ingredients.

It is advised to perform dermal absorption studies in the risk assessment process. In the absence of experimentally determined dermal absorption, **50%** bioavailability as default value of the substance is used. This conservative value may also be used in cases where only 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 from the calculations (*e.g.* opinion on Polyaminopropyl Biguanide (PHMB) - Submission III, SCCS/1581/16) 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 that are poorly soluble in water is important in the receptor fluid of an *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.

Where studies correspond to all of the basic requirements of the SCCS, the mean +1SD will be used for the calculation of the MoS. In case of significant deviations and/or very high variability, the mean + 2SD may be used. Where the deviation is too high, the study is not accepted and is excluded.

Especially for substances intended to be used as UV-filters, studies have been submitted to the SCCS using damaged skin (*e.g.* SCCS/1594/18; SCCS/1546/15). So far, there is no standard protocol for the investigation of dermal absorption through damaged skin, or a common understanding of "damaged skin" (wounded, physically damaged, sunburnt, etc.). Therefore, the SCCS prefers study results obtained with intact skin. Information from damaged skin can only be considered as supporting information.

It should be noted that when experimental values have been derived from a limited number of data points (N), standard deviation is calculated using 'N'. Only in cases where the number of data points is > 30, can 'N-1' be used.

c. Substances with very low dermal absorption

A retrospective study of the Annex 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 may be indicative of very low dermal absorption (Ates *et al*., 2016). For dealing with data on very low dermal absorption, see Section 3-6.10.

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

3.3.5.1.2 ABSORPTION AFTER INGESTION

For products intended for oral use, like toothpastes and mouthwashes, inevitably some amount will be ingested. If no experimentally derived data are provided, the SCCS will take the conservative absorption value of 100%.

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 cell culture model for oral 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, Fredlund *et al.,* 2017). 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, Beloqui *et al.* 2016). Study of the predictive capacity of two *in vitro* cellular systems- the Caco-2/ATCC parental cell line and the Caco-2/TC7 clone 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). The model has been a subject of improvement (Shah *et al.* 2014, Takenaka *et al.,* 2017, Di Marco *et al.*, 2017).

3.3.5.1.3 INHALATION

Cosmetic ingredients might be inhaled as gases, vapours, (liquid) aerosols or powders and enter the respiratory tract. The physical form of the ingredient plays a decisive role in the absorption process. Further, absorption *via* inhalation is governed by respiratory patterns and the physiology of the respiratory tract, which consists of the nasopharyngeal, the tracheobronchial and the pulmonary regions.

Gases and vapours are absorbed in the pulmonary region. However, if gases are reactive or very water soluble, they might not reach the pulmonary region due to reaction with cell

surface components of the naso- or tracheobronchial region or due to solution into the aqueous mucus layer of the respiratory tract (eventually followed by out-partitioning). Thus, hydrophilic vapours/gases are more prone to be removed from the upper respiratory tract whereas lipophilic substances are more likely to reach the deep lung. There, absorption into the bloodstream may occur when the molecule is sufficiently lipophilic to dissolve in the lipophilic alveolar mucus and to cross the alveolar and capillary membranes.

The rate of absorption of a gas into the circulation is governed by the blood to gas partition coefficient (the ratio of the concentration of a chemical in blood and the concentration of the chemical in the gas phase).

Once deposited in the lung, (partially) soluble particles dissolve (partially) in the lining fluid (mucus layer) of the epithelium where inert particles might form non-dissolved but colloidal suspensions. For further considerations of particle behavior refer to SCCS/1484/12:

Guidance on the Safety Assessment of Nanomaterials in Cosmetics (under revision).

If information on the extent of inhalation absorption is available from experimental studies and/or physico-chemical parameters, this information is used. However, if no data are presented, the SCCS considers that for the calculation of inhalation exposure an absorption of **100%** should be used.

3-3.5.2 DIFFERENCES IN METABOLISM FOR DIFFERENT ROUTES

3-3.5.2.1 SYSTEMIC METABOLISM

Metabolism of xenobiotic substances in mammals mainly occurs *via* phase I and/or phase II reactions mediated by xenobiotic metabolising enzymes (XMEs). This can also involve active transport of substances in (Phase 0) and/or out of the cells (Phase 3). 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 so that they can be better eliminated *via* bile or urine, by conjugation mainly with glutathione, glucuronic acid or sulfate. In most cases, phase I metabolites that may be reactive are also inactivated by these conjugation reactions.

Metabolism of xenobiotic substances 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 subfamilies 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 3-5.1). 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. Although 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).

In mammals, expression and regulation of XMEs depend on many factors, including genetic factors (polymorphisms), external causes (*e.g.,* enzyme inducers or inhibitors), individual factors such as gender, age, nutrition, health status (disease), pregnancy and several other factors. These potential individual differences are considered in risk assessment by the use of an ***intraspecies* default factor** for toxicokinetics (including metabolism) (see Section 3-5.1). 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 metabolic capacity per gram of tissue. In addition to quantitative differences in metabolic capacity there are also major differences in the constitutive expression and regulation of XMEs between mammalian liver and extra-hepatic tissues including skin (Oesch *et al.*, 2007; Gundert-Remy *et al.*, 2014; Oesch *et al.*, 2014).

Therefore, in some cases, when an XME isoenzyme form is not active in rodent 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 is not a regular requirement for SCCS safety evaluation, such data is helpful and sometimes required to complete 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 and increasingly from human sources.

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 development (Anton *et al.,* 2015; Baptista *et al*., 2016; Fang and Eglen, 2017; Chen *et al.,* 2018).

Under *in vitro* conditions, first pass effects cannot be captured.

Extrapolation from *in vitro* metabolism data to the *in vivo* situation 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 3-3.5.3). Often, *in vivo* data from laboratory animals, or even more from humans, is helpful or even indispensable in order to clarify if or to which extent relevant metabolites are formed (see OECD 417 on toxicokinetics).

Because of the species differences of XMEs, human *in vivo* data are the gold standard, however, it should be considered as the last resort and in view of the restrictions mentioned in Section 3-4.7 and the Memorandum on the use of human data (SCCS/1576/15).

Some examples including human toxicokinetic data can be found in SCCS Opinions such as for zinc pyrithione (SCCS/1512/13), cyclopentasiloxane D5 (SCCS/1549/15), phenoxyethanol (SCCS/1575/16) and salicylic acid (SCCS/1601/18). 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 (*e.g.* p-phenylenediamine (A7) SCCS/1443/11).

3-3.5.2.2 DERMAL METABOLISM

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 a 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. Phase II reactions in skin apparently play a greater role than phase I reactions of which the metabolic capacity 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 (Oesch *et al.*, 2007; SCCP/1171/08, Oesch *et al.*, 2014; Gundert-Remy *et al.*, 2014).

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 (*e.g.* 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; Wiegand *et al.* 2014). These models are now well-established, but additional work is still necessary as none of these skin models has yet been officially validated for metabolism.

These skin models may help in the future to clarify important questions *e.g.* oxidative bio-activation of prohaptens to haptens (Bergström *et al.*, 2007; Karlberg *et al.*, 2008, 2013, SCCS/1459/11, Urbisch *et al*., 2015 and 2016).

3-3.5.2.3 LUNG METABOLISM

The lung is a complex organ comprised of anatomically different parts (trachea, bronchi, bronchioli and lung alveoli) accommodating a large number of different cell types which might contribute to xenobiotic metabolism. Similar to skin, also in lung the expression of xenobiotic metabolizing enzymes is lower compared to liver. Nevertheless, there are certain metabolising enzymes which are preferentially expressed in the lung (*e.g.* CYP2A13, CYP2F1). Both functionalising and conjugating enzymes have been identified mainly in bronchiolar epithelium but also in pneumocytes, alveolar macrophages, Clara cells, respiratory epithelium or serous cells. CYP enzymes involved in xenobiotic metabolism have been identified in lung tissues from different species including humans (overview, Gundert *et al.,* 2014).

They can vary considerably between humans. Amongst conjugating enzymes, glutathione S-transferases (GSTs), uridine diphosphate glucuronosyltransferases (UGTs) and arylamine-N-acetyltransferases (NATs) and in part also their local distribution in the lung have been identified. Other enzymes present in lung are epoxide hydrolases or certain transporters (Multidrug Resistance Proteins MDR1 and MRP1 or Breast Cancer Resistance Protein BCRP) (Gundert-Remy *et al.,* 2014).

3.3.5.3 PBPK MODELLING

PBPK models are quantitative descriptions of the ADME of chemicals in biota based on interrelationships among key physiological, biochemical and physicochemical determinants of these processes (WHO, 2010).

These models are not only used to translate external exposures into an internal (target) dose in the body, but are also developed to help with:

- 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 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 PBPK model). The use of estimated values in further modelling might, however, increase uncertainties associated with a model.

The PBPK model should be capable of predicting the observed basic pharmacokinetics of the chemical (parent compounds or metabolites) before the model can be used for simulations of specific scenarios. Moreover, the acceptable prediction of dose metric should follow the acceptance criteria as indicated in the WHO guidance (IPCS, 2010) *i.e.* the ratio between simulated and observed data should be on average within a factor of 2. If the ratio between simulated and observed data (parent compounds and/or metabolites) is not within a factor of 2, it will be necessary to refine and update the model with further ADME data.

If a metabolic scheme is available, evaluation on how well the model describes the respective metabolic/biochemical processes (number of metabolites, metabolites tree) should be performed.

Sensitivity analysis is an important component of model verification, especially for uncertain parameters with a high potential to influence the outcome of the simulation. A sensitivity analysis needs to be performed for all parameters. It provides a quantitative evaluation of how input parameters influence the dose metrics or other model output of relevance to the risk assessment, or to the problem as defined at the beginning (WHO/IPCS, 2010).

Note that: Sensitivity analysis results are expressed as absolute values of a normalised coefficient and are:

- High: ≥ 0.5
- Medium: 0.2 ≤ medium< 0.5
- Low: 0.1 ≤ low< 0.2

Uncertainty analysis must be performed by the Applicant. It evaluates the impact of the lack of precise knowledge of parameter values and model structure on dose metric simulations (WHO/IPCS, 2010). For parsimony, uncertainty analysis could be limited to the parameters identified through the sensitivity analysis as the ones that have the highest likelihood to affect the result of the model calculations.

The notion of uncertainty encompasses both true uncertainty (*i.e.* in model parameter value) and variability (*i.e.* from population variability). Variability refers to inherent heterogeneity that is distributed within a defined population, such as body weight. In contrast, true uncertainty refers to a parameter that has a single value, which cannot be known with precision due to measurement or estimation error, such as partition coefficient.

The level of uncertainty is determined based on the ratio of the 95th percentile (P95) over the median value (P50) for the selected dose metric *i.e.*, Area Under the Curve (AUC), Maximum Concentration (Cmax), etc.

Uncertainty analysis results are either summarised as having a high uncertainty (value could be a factor of 2 or higher); a medium uncertainty (value could be a factor between 0.3 and 2) or a low uncertainty (value could be a factor of 0.3 or lower).

The outcome of sensitivity and uncertainty analyses might inform the reliability of a model to provide dose metric predictions of use in risk assessment, as illustrated in **Figure 3** (WHO/IPCS, 2010).

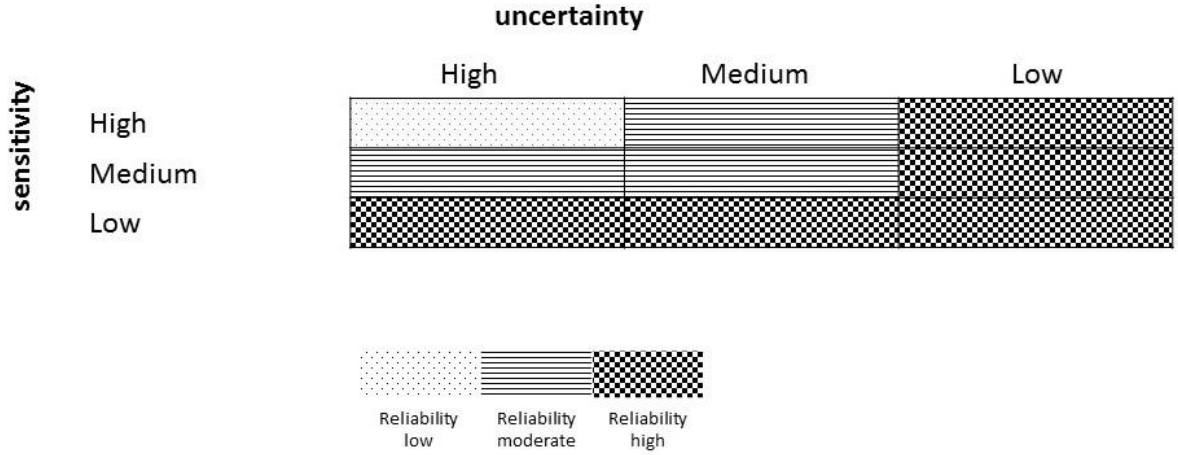


Figure 3: Illustration of the role of sensitivity and uncertainty analyses in determining the reliability of PBPK model predictions of dose metrics for safety evaluation (WHO/IPCS, 2010)

Note that uncertainty and sensitivity analysis are generally necessary for any type of model calculation.

The reliability of the model predictions of dose metrics for the safety evaluation, where feasible, are based on the level of sensitivity of the predictions to the model parameters and the level of uncertainty of the parameter values.

If the highly sensitive parameters are also the ones that are highly uncertain, then the reliability of the model would be questionable (WHO/IPCS, 2010).

When estimated data from PBPK models are submitted to SCCS which are intended to be used for MoS calculation, *i.e.* for quantitative safety evaluation, then it should also be demonstrated that the 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 and information about software used should be provided – preferably in a tabular form.

In conclusion, SCCS will use data from PBPK models for quantitative risk assessment only if sufficient details (see below) are provided so that the calculations can be evaluated. Otherwise, the data may only be used as supporting information. In this respect, the following are needed:

1) Model structure and characterisation that involves the development of conceptual and mathematical descriptions of the relevant compartments of the human or animal body as well as the exposure and metabolic pathways related to the chemical under study.
2) Model parameterisation that involves obtaining quantitative estimates of measures of the mechanistic determinants (*e.g.* anatomical, physiological, physicochemical, biochemical parameters);
3) Mathematical and computational implementation
4) Model simulation, *i.e.* simulation of the kinetics;
5) Model evaluation and validation that involves comparison of the *a priori* predictions of the PBPK model with experimental data as well as conducting uncertainty, sensitivity and variability analyses.

It should be noted that PBPK modelling has usually been based on experimental data, often animal data, to build up the model. It needs to be stressed that such modelling results will only be acceptable if data from animal tests have been used within the relevant regulatory restrictions.

3-3.5.4 CALCULATION OF THE SYSTEMIC EXPOSURE DOSE (SED)

The systemic dose can be calculated following different tiers. In a first tier, the SED is calculated deterministically from the first tier conservative external exposure estimates by multiplication with a conservative point value for the absorption fraction. Normally, the major route of exposure will be *via* the skin. Therefore, the following equations specifically treat the calculation of first tier exposure *via* skin but can be adapted for other routes accordingly. Higher tier calculation of the SED can be derived *e.g.* from external exposure distributions derived with probabilistic models (see Section 3-3.4).

<u>Calculations of the SED</u>

There are two ways of calculating the SED, depending on the way the dermal absorption of a compound is reported:

-it is preferably 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 3** in Section 3-3.4.2) to estimate the systemic availability of the substance.

-it may also be based on the **percentage** dermally absorbed. This depends on the amount of finished product applied on the skin (see **Table 2A** and **Table 2B** in Section 3-3.4.2 for default values per product type).

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

For calculating the SED, the skin surface has to be taken into account that should be treated with the finished cosmetic product containing the substance under study, as well as the frequency of product 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).

$$SED = \frac{DA_a \times 10^{-3} \times SSA \times f}{bw}$$

Where:

SED (mg/kg bw/d)	Systemic Exposure Dose
DAa (µg /cm^2)	Dermal Absorption as amount per surface, resulting from an assay under in-use mimicking conditions
SSA (cm^2)	Skin Surface Area expected to be treated with the finished cosmetic product (see **Table 3** in Section 3-3.4.2 for SSA values per product type)
f (day^{-1})	Frequency of application of the finished product (f ≥1)
bw (kg bw)	human body weight (default value: 60 kg)

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.

$$SED = E_{product} \times \frac{C}{100} \times \frac{DA_p}{100}$$

Where:

SED (mg/kg bw/day)	Systemic Exposure Dose
$E_{product}$ (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, **Tables 2A** and **2B**, Section 3-3.4.2).
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

If the actual number of applications differs from the standard application frequency assumed for deriving the default values in **Tables 2A** and **2B**, the SED for the respective product category will have to be adapted accordingly.

3-3.5.5 AGGREGATION OF THE SYSTEMIC DOSE

If all product categories have the same uptake rate or fraction, the aggregated SED can be calculated by multiplying the route-specific aggregate external exposure with this uptake rate or fraction. If some product categories are taken up at a different rate than the others, the single external exposures need to be multiplied with the specific uptake rates, and then aggregated.

If aggregation should be done over routes, the route-specific SEDs can be added up. In some cases (like *e.g.* when metabolism is different for the different routes) a PBPK model needs to be applied for aggregating over routes.

3-4 RELEVANT TOXICOLOGICAL STUDIES ON COSMETIC INGREDIENTS

3-4.1 INTRODUCTION

The SCCS has been closely following the progress made with regards to the development and validation of alternative methods and updated its NoG on a regular basis.
Besides the validated alternatives the SCCS may accept, on a case-by-case basis, as well methods that are scientifically valid as new tools (*e.g.,* "-omics" technology) for the safety evaluation of cosmetic substances. Such valid methods may not have necessarily gone through the complete validation process, but the Committee may consider them acceptable when there is 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).

Whereas the often used terminology of "alternative test methods (ATMs)" only covers test methods and not for example *in silico* methodology, the term NAMs is more general.

Non-animal Alternative Methods

The need for non-animal alternative methods for chemical hazard assessment is much more important for compliance with the Cosmetics Regulation than other regulatory frameworks in Europe. At the origin are the testing and marketing bans and the obligation to only use validated replacement alternatives.

The main alternative methods not using animals and relevant to cosmetic hazard assessment include *in vitro, in chemico and in silico* methods, and read-across, as well as the use of combinations thereof. It is therefore advisable that, before any testing is carried out for safety evaluation, all information on the substance under consideration should be gathered from different available means.

3-4.2 *IN SILICO* ASSESSMENT OF TOXICOLOGICAL HAZARD

The *in silico* models and tools are based on principles, rules and structural alerts that have been derived from the relationship(s) between chemical structure and toxicity of a group of related substances. The methods have gained a special importance as they offer a rapid, cost-effective, and ethical alternative to animal testing of chemical toxicity.

The field of *in silico* toxicology has undergone a lot of scientific developments over the past few decades with the availability of large property/effect databases, powerful data-mining tools, diverse statistical algorithms and soft-computing techniques. As a result, a number of *in silico* models and tools is now available.

These include predictive computational models based on Structure-Activity Relationship (SAR) and Quantitative Structure-Activity Relationship (QSAR), as well as computational tools for read-across of data from structurally or functionally similar substances to a target (untested) substance, and toxicity expert systems that combine the rules, structural alerts and/or (Q)SAR models.

This has also led to the development of hybrid models that derive toxicity estimates from a combination of knowledge-based rules and statistically-derived models (Benfenati, 2012). The currently available models and tools cover a wide variety of chemical types and many of the key toxicological endpoints that are required for chemical risk assessment.

In silico Toxicity Models

The toxicity estimates derived from a non-testing approach, such as a (Q)SAR model, can only be as much reliable as the chemical and toxicological data and the rules/algorithms used to build it, the degree to which it was tested and validated, and whether the query substance is covered within its applicability domain (*i.e.* the model's prediction space). Because each model/system has a finite number and type of chemical structures behind it, there will always be a limit to its applicability domain. In this regard, an *in silico* model/system is only considered appropriate for regulatory use if it has been developed in accordance with the stringent quality criteria and the validation principles laid down by the OECD in 2004 (www.oecd.org/chemicalsafety/risk-assessment/37849783.pdf). This means that a (Q)SAR model/system not only needs to have been based on high quality chemical and toxicological data, but it should also address a defined endpoint, be based on ambiguous rule(s)/ algorithm(s), clearly define the applicability domain, provide appropriate measures of the goodness-of-fit, robustness and predictivity, and where possible, also provide a mechanistic interpretation.

A few of such models/systems are available in the form of both commercial and free-access software platforms that may be considered for use in regulatory risk assessments. The EU project ANTARES has carried out assessment of the validation characteristics of a range of (Q)SAR models for various (eco)toxicological and environmental endpoints relevant to data requirements under the chemical legislation REACH (Registration, Evaluation, Authorisation and restriction of Chemicals). The project's website (www.antares-life.eu/) provides a list of the currently available free-access and commercial *in silico* models and tools.

ECHA (2016) has published a document on how to use and report results from QSAR models.

Examples of the free-access *in silico* systems include[2] the OECD QSAR ToolBox that provides a versatile suite of programs for the prediction of different toxicity endpoints based on categorisation, (Q)SAR models, and read-across (www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm). Other examples of free-access *in silico* models/systems include Hazard Evaluation Support System (HESS) for the assessment of repeated-dose toxicity (www.nite.go.jp/en/chem/qsar/hess-e.html); and the expert systems such as Cramer Decision Tree (Lapenna and Worth, 2011) that is based on structural alerts and expert knowledge; the Benigni-Bossa Rule Base (Benigni *et al.,* 2008) that is based on structural alerts and QSARs for genotoxicity and carcinogenicity; the Toxicity Estimation Software Tool (T.E.S.T.) that is based on an ensemble of QSAR models (www.epa.gov/chemical-research/toxicity-estimation-software-tool-test); and the VEGA QSAR platform that is based on (Q)SARs and other *in silico* tools (www.vega-qsar.eu).The JRC maintains an inventory of available QSAR models (https://eurl-ecvam.jrc.ec.europa.eu/databases/jrc-qsar-model-database).

A QSAR Model Reporting Format (QMRF) has also been developed by the JRC and EU Member State authorities for summarising and reporting key information on QSAR models, including the results of any validation studies. The information is structured according to the OECD validation principles.

The International Cooperation on Cosmetics Regulation (ICCR), a platform of regulators and cosmetics industry from the EU, the US, Japan, Canada and Brazil, has reviewed the use of *in silico* methods for safety evaluation of cosmetic ingredients.

[2] Mention of any *in silico* model/system in this document does not constitute an approval of its quality, or recommendation for use by the SCCS.

The ICCR report (2014) has concluded that the current use of *in silico* approaches for safety evaluation of cosmetic ingredients is largely limited to internal decision making both at the industry and at the regulatory levels, and that they have not yet been adopted as a mainstream alternative to testing methods.

This is because different models and systems may have been built using different datasets, rules and/or algorithm(s), and therefore interpret chemical structures and toxicological data in different ways. Each model/system also reflects a different level of uncertainty and variability associated with the data used for developing it, the modelling process used, and the differences in the applicability domains. In view of this, a high quality *in silico* model/system needs to provide not only the toxicity estimates but also a measure of uncertainty in the results.

The SCCS has published a Memorandum on the use of *in silico* methods for assessment of chemical hazard (SCCS/1578/16). The memorandum has identified a number of limitations and barriers in regard to the use of *in silico* models/systems in regulatory risk assessment of chemicals. These include the fact that regulatory risk assessors use data mainly from 'validated' methods for risk assessment, they also consider that virtually none of the currently available *in silico* models/systems carries an authoritative 'validation' tag. Other limitations of *in silico* methods include inability of most of the free-access models/systems to make precise estimates of the toxicity of different stereo-isomers of chemical substances, inorganic substances, and some other types of materials (*e.g.* nanomaterials). Despite such limitations, of the use of 'valid' *in silico* methods, models and tools that are currently available may provide supporting evidence as part of weight of evidence for risk assessment of cosmetic ingredients. The outcome of *in silico* assessment can also provide useful insights to the hazard identification that can guide planning of *in vitro* testing.

Read-across

A number of computational tools have been developed that allow the selection of closely-similar analogues for data read-across on the basis of structure-activity principles and rules (https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across).

In this regard, *in silico* models based on k-Nearest Neighbour (kNN) algorithm identify analogous compounds that are most closely-related to the target compound. Examples of *in silico* platforms that incorporate kNN based models include VEGA and TEST. A number of other programs have been designed specifically for read-across (Patlewicz *et al.* 2017). Examples include ToxRead (www.toxread.eu), which also shows chemical analogues in a graphic format, provides reasoning for relevance of the effect to the target compound, and a description of the statistical importance of each rule.

The OECD toolbox also provides a means for read-across from its comprehensive databases and/or additional datasets that can be added by the users. Similarly, AMBIT (http://cefic-lri.org/lri_toolbox/ambit/) and Toxmatch (https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology/qsar_tools/toxmatch) also provide useful means for identifying similar substances and read-across.

It needs to be emphasised that read-across should be carried out using appropriate systems/tools that allow impartial selection of closely-related analogues on the basis of structure-activity based rule/algorithm. This is of utmost importance to avoid any subjective selection and use of only a few analogues selected randomly on the basis of personal choices or judgement.

In summary, whilst *in silico* models provide useful methods that do not use animals for deriving estimates of toxicity of untested compounds, each model has certain limits regarding the reliability of the results as well as the coverage of different chemical types and toxicological endpoints. Therefore, the use of a single *in silico* model/system is generally not adequate, and more than one relevant model/system should be used to increase confidence in the derived toxicity estimates. The *in silico* results are also more useful for hazard assessment when they are integrated with other sources of evidence (*e.g. in vitro* results) into the overall weight of evidence (WoE) (SCCS/1578/16; EFSA, 2017a).

Thus, whenever possible, *in chemico* (*i.e.* grouping and other chemical analogy approaches) and *in silico* (*i.e.* QSAR) methods should be applied to derive estimates of toxicity before any experimental testing is considered. It should, however, also be appreciated that the use of *in silico* models and interpretation of the results requires expert judgement and therefore must not be treated as the outcome of a 'black box' technology.

3-4.3 ADVERSE OUTCOME PATHWAY (AOP)

An Adverse Outcome Pathway (AOP) is an analytical construct that describes a sequential chain of causally-linked key events at different levels of biological organisation that lead to an adverse health or ecotoxicological effect. An AOP starts with a molecular initiating event (MIE), which is the chemically-induced perturbation of a biological system at the molecular level, which eventually leads to a specific adverse outcome. The MIE triggers a sequence of key events (KEs) that occur at the cellular or organ level and are causally linked to the adverse outcome. The AOP framework has been taken up by the OECD, providing a website to follow new developments on this subject (https://aopwiki.org/). OECD 2012a, 2013b give guidance on how to document, present and assess the relevance and adequacy of an AOP. The AOP concept has been successfully applied to a number of human-relevant toxicological endpoints including skin sensitisation (OECD, 2012b) (see Section 3-4.3). The quantitative aspect is, however, still a weak point.

AOPs can be used to support the development of Integrated Approaches to Testing and Assessment (IATA) and Defined Approaches (DA) (OECD, 2012a, 2012b, 2013b, 2014b; 2017a; Tollefsen *et al.*, 2014).

An **IATA** is a pragmatic approach that exploits and weighs existing information, including human data and exposure information, alternative methodologies, such as *in chemico* and *in vitro* assays, and tailored strategies for the purpose of chemical evaluation with applications in risk assessment (Patlewicz *et al.*, 2015; Tollefsen *et al.*, 2014). While IATAs provide a platform for data integration and a means for targeted testing for a specific purpose, it is not necessarily framed by a mechanistic rationale. AOPs could be used to provide this mechanistic basis and thus to identify data gaps or to contextualise a diverse range of existing data (Delrue *et al.*, 2016, OECD 2017a, Tollefsen *et al.*, 2014).

A **DA** consists of a fixed-data interpretation procedure applied to data generated with a defined set of information sources to derive a result that can either be used on its own, or together with other information sources within an IATA, to satisfy a specific regulatory need (OECD, 2017a).

3-4.4 ACUTE TOXICITY

The term **acute toxicity** is used to describe the adverse effects, which may result from a single exposure to a substance. Exposure relates to the oral, dermal or inhalation routes.

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

3-4.4.1 ACUTE ORAL TOXICITY

A. <u>NAMs</u>

- The only validated *in vitro* method existing at present for acute oral toxicity (EURL ECVAM endorsed) is the 3T3 NR (neutral red) uptake test, applicable for non-classified chemicals, based on a cut-off of LD50>2000 mg/kg bw (JRC, 2013).

B. <u>In vivo</u>

- The *in vivo* acute oral toxicity test was originally developed to classify the hazard of chemicals based on their LD_{50} value. LD_{50} values are also used to trigger the labelling of compounds with respect to acute toxicity (2008/1272/EC).

The original test method (EC B.1, OECD 401) has been replaced by alternative methods. These still are animal tests. Therefore, results generated *via* these tests are only allowed when performed before the testing and marketing bans were fully applied, or if the data were obtained in order to be in compliance with other (non-cosmetics) legislation *e.g.* REACH. The following refinement/reduction tests have been validated and consist of:

- 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.

3-4.4.2 ACUTE DERMAL TOXICITY

No validated non-animal alternatives for the *in vivo* acute dermal toxicity test (EC B.3,) are currently available, however the updated OECD guideline 402 for the **fixed dose procedure** is more in line with the 3R's principles.

3-4.4.3 ACUTE INHALATION TOXICITY

Currently no validated non-animal alternative exists for the replacement of the *'in vivo'* acute inhalation toxicity test (OECD 403). The latter was revised in 2009 (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 guideline of the **fixed concentration procedure** by inhalation.

3-4.5 SKIN CORROSION AND SKIN IRRITATION

3-4.5.1 SKIN CORROSION

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).
Corrosivity could occasionally occur after a manufacturing error or product misuse. A cosmetic substance that has the intrinsic property to be corrosive is not necessarily excluded for use in cosmetics. An example is potassium hydroxide KOH, the corrosivity of which depends on the final concentration, the pH, the presence of "neutralising" substances, the excipient used, the exposure route, etc.

A. NAMs

For **skin corrosion** testing, at present, there are three test guidelines on *in vitro* replacement alternatives:

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), including the Corrositex® test method, which has not been adopted in the European legislation.

B. *In vivo*:

The OECD 404 test is not allowed anymore for cosmetics and their ingredients. Data obtained from the *in vivo* skin corrosion/dermal irritation test should only be provided when already available for a test performed before the animal testing ban or if the data were obtained for the purpose to be in compliance with other (non-cosmetic) legislations.

3-4.5.2 SKIN IRRITATION

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).

A. NAMs

For **skin irritation** testing, at present, there is one test guideline on *in vitro* replacement alternatives:
The Reconstructed Human Epidermis (RhE) Test Method (OECD 439) includes 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.

These 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 OECD Test Guidelines (TGs) 430, 431 and 435, the revised TG 439 (July 2015) also includes performance standards developed by EURL-ECVAM to facilitate the validation and assessment of possible future RhE-based test methods. 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). In order to obtain better sensitivity, while maintaining similar specificity, a second endpoint, interleukin-1α (IL-1α) production, has been suggested.

The *in vitro* test for skin irritation has been found useful by the SCCS for the testing of cosmetic ingredients. However, when reducing substances, hair dyes and colourants are present, which could interfere with the formazan colour evaluation (Lelièvre *et al.* 2007, SCCS/1392/10), HLPC separation prior to quantification should be carried out (SCCS/1392/10) for coloured and non-coloured test chemicals (Alépée *et al.,* 2015). OECD 431 and 439 support this methodology.

OECD has developed a Guidance Document No. 203 on an IATA for skin corrosion and irritation (OECD, 2014b). The Guidance Document has two aims: i) to propose an integrated approach for replacing the strategy provided in the *in vivo* test guideline (OECD 404) and ii) 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.

B. *In vivo*:

The OECD 404 test is not allowed anymore for cosmetics and their ingredients. Data obtained from the *in vivo* skin corrosion/dermal irritation test should only be provided when already available for a test performed before the animal testing ban or if the data were obtained for the purpose to be in compliance with other (non-cosmetic) legislations.

3-4.6 SERIOUS EYE DAMAGE AND EYE IRRITATION

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).

An IATA of this endpoint is available. The evaluation of serious eye damage and eye irritation should be carried out according to OECD Guidance (OECD 263).

A. NAMs

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 OECD *in vitro* test guidelines adopted, which are subdivided in 3 groups (a, b, c). These are:

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

1) The Bovine Cornea Opacity Permeability (BCOP) test method measures 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 evaluates the ability of a test chemical to induce toxicity in an enucleated chicken eye (EC B.48, OECD 438). 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 that induce serious eye damage {Cat. 1 according to the United Nations Globally Harmonised System of Classification and Labelling of Chemicals (UN GHS) definitions}.

(ii) Chemicals that do not require 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 may be useful in providing supportive evidence (JRC website 2016, 2017).

b) ***cytotoxicity and cell function-based in vitro tests***, including 2 OECD guidelines:

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 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, validated by ECVAM in 2009, uses a pH-meter to detect changes in acidity in a sub-confluent monolayer of adherent mouse L929 fibroblasts. A draft OECD TG exists on its use 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. The CM test method cannot exclude mild eye irritant potential and only applies to water soluble substances and mixtures as well as non-water soluble solid, viscous chemicals or suspensions that maintain uniformity during analysis time. This methodology has in particularbeen used in the USA.

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

c) ***reconstructed human tissue (RhT)-based test methods***:

5) The Reconstructed Human Cornea-like Epithelium (RhCE) test method (OECD 492, B.69), evaluates the ability of a test chemical to induce cytotoxicity *via* the MTT assay. The adopted TG includes the HPLC/UPLC technique for measuring the formazan formation, for the evaluation of chemicals which may interfere with MTT-formazan measurement by direct reduction of MTT or colour interference. 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, which uses a commercially available non-transformed human-derived epidermal keratinocyte model, is covered by OECD 492 and B.69. Currently, the available replacement alternatives for serious eye damage and eye irritation testing cannot identify any mild eye irritancy potential.

So far, neither a single *in vitro* assay nor a testing battery has been validated as a stand-alone replacement for the *in vivo* test. Different decision trees for eye irritation were put forward (McNamee *et al.*, 2009), but none can identify mild, moderate or non-eye irritancy (McNamee *et al.* 2009, Scott *et al.,* 2010). An overview of current techniques for ocular toxicity testing is presented by Wilson *et al.* (2015) and Lotz *et al.* (2015). New test systems are under development using stem cells. These could generate new alternatives for *in vitro* ocular toxicity testing (Aberdam *et al.,* 2017).

B. In vivo

The *in vivo* test (OECD 405, EC B.5) has been subject to refinement and reduction measures. It was also indicated that histopathology is an additional endpoint in ocular safety testing. The latest update has mainly focused on the use of analgesics and anesthetics. It is the only *in vivo* test method to assess the potential of a substance to cause acute serious eye damage / irritation.

The results from this test should be provided if already available from a test that was performed before the animal testing ban or if data were obtained for the purpose of compliance with other (non-cosmetic) legislations, *e.g.,* REACH.

3-4.7 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. Once a person is sensitised, subsequent skin exposure at a sufficiently high concentration can provoke allergic contact dermatitis.

A. NAMs

In the last years, several NAMs have been developed, validated and regulatory accepted (Ezendam *et al.*, 2016, Hoffmann *et al.,* 2018) that address different KEs of the skin sensitisation AOP (OECD, 2012) (**Figure 4**) (see introductory part of Section 3-4.3). The MIE of this AOP is covalent binding of the chemical to skin proteins, leading to an immunogenic hapten-carrier complex. The MIE triggers KE2, keratinocyte activation, and KE3, dendritic cell activation. Subsequently, the activated and differentiated dendritic cells migrate to the draining lymph nodes and present their small peptides of the hapten-carrier complex to the T cells. This leads to KE4: T cell activation and proliferation creating a pool of memory T cells, ultimately leading to skin sensitisation (adverse outcome).

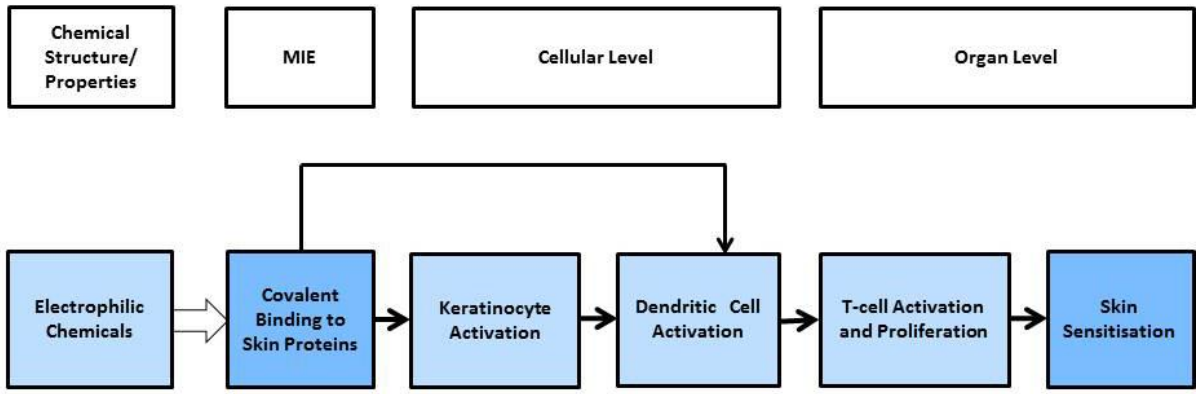


Figure 4: AOP Covalent Protein binding leading to Skin Sensitisation (taken from https://aopwiki.org/wiki/index.php/Aop:40); MIE: molecular initiating event.

The skin sensitisation AOP is used in the development of IATA and DA that combine multiple NAMs to predict skin sensitisation potential and potency (Ezendam *et al*., 2016, Kleinstreuer *et al.,* 2018, OECD 2017b). **Table 5** provides an overview of the NAMs for skin sensitisation that are currently included in the OECD and/or EU test guideline program. The OECD has clustered test methods that address the same KE of the AOP in one test guideline.

Several NAMs for skin sensitisation are still being developed or validated (OECD, 2017b, Ezendam *et al.,* 2016, Hoffmann *et al.,* 2018) (**Table 5**). Two of these have been included in the OECD test guidelines work plan: the SENS-IS and Genomic Allergen Rapid Detection test method (GARD). SENS-IS is an *in vitro* model that measures KE2 by assessing gene expression profiles in a human skin model (Episkin® RhE). SENS-IS allows categorisation of sensitisers into potency categories (Cottrez *et al.*, 2015). The SENS-IS assay has been validated in an industry-led study (Cottrez *et al.*, 2016) and is currently being evaluated by EURL-ECVAM. The GARD is an *in vitro* model that measures KE3 using gene expression profiling in the MUTZ-3 cell line (Johansson *et al.*, 2011, 2014). The validation study is currently ongoing.

There are currently no NAMs available in the OECD test guideline program that address KE4 (T cell activation and proliferation) (van Vliet *et al.*, 2018).

Table 5: NAMs for the assessment of skin sensitisation

AOP KE covered	***OECD test guideline/ EU test method***	***Test method***
MIE (KE1): covalent binding to skin proteins	OECD 442C / EC B.59 *In chemico* skin sensitisation	Direct Peptide Reactivity Assay (DPRA)
KE2: keratinocyte activation	OECD 442D / EC B.60 *In vitro* Skin Sensitisation Assays addressing the KE on keratinocyte activation	ARE-Nrf2 Luciferase KeratinoSens™ Test Method The ARE-Nrf2 luciferase LuSens test method
KE3: dendritic cell activation	OECD 442E / EC B.72 *In vitro* Skin Sensitisation Assays addressing the KE on activation of dendritic cells.	Human Cell Line Activation test (h-CLAT) U937 Cell line Activation Test (U-SENS™) Interleukin-8 Reporter Gene Assay (IL8-Luc assay)

MIE: molecular initiating event; AOP: adverse outcome pathway; KE: key event

The currently available NAMs for skin sensitisation address only one single key event of the AOP. Furthermore, the individual test methods have some known technical limitations, such as no or limited metabolic capacity. Still some pre- and pro-haptens can be detected (Patlewicz *et al.* 2016). For these reasons, a single alternative method cannot be used as a stand-alone assay for hazard identification or to sub-categorise skin sensitisers into subcategories extreme, strong or moderate. It is therefore recommended to combine these methods and other information sources (*e.g.*, *in silico* tools) in an integrated approach, such as a DA or IATA. Examples on how the individual test methods for skin sensitisation can be combined can be found in Annex 1 of OECD Guidance Document No. 256 (OECD, 2017b) or in recent publications (Ezendam *et al.,* 2016, Kleinstreuer *et al.,* 2018). Some of these NAMs only provide information on hazard, whereas others provide information on potency as well. Additional work is ongoing to determine how *in vitro* concentration response data can be exploited in integrated approaches for human potency prediction.

B. <u>In vivo</u>

-Three regulatory accepted *in vivo* laboratory animal test methods have been used to evaluate the potential of a substance to cause skin sensitisation, the Local Lymph Node Assay (LLNA), the Magnusson Kligman Guinea Pig Maximisation Test (GPMT) and the Buehler test (**Table 6**). The GPMT and Buehler tests are able to provide results on induction and elicitation; the LLNA and its variants only address induction.

Table 6: *In vivo* laboratory test methods for evaluation of skin sensitisation

Species	***Test method***	***Endpoint***	***Guideline***
Mouse	LLNA (radioactive method)	Cellular proliferation SI≥3	OECD 429, EC B.42
Mouse	LLNA:DA (non-radioactive method)	Cellular proliferation SI≥1.8	OECD 442A, EC B.50
Mouse	LLNA: BrdU-ELISA (non-radioactive method)	Cellular proliferation SI≥1.6	OECD 442B, EC B.51
Guinea pig	GPMT	Score of erythema and swelling	OECD 406, EC B.6
Guinea pig	Buehler test	Score of erythema and swelling	OECD 406, EC B.6

LLNA: Local Lymph Node Assay; GPMT: Guinea Pig Maximisation Test; SI: Stimulation Index
LLNA:DA: nonradiolabelled LLNA, modified by Daicel Chemical Industries
LLNA:BrdU-ELISA: nonradioactive modification of LLNA based on cell proliferation measured by 5-bromo-2'-deoxyuridine

As presented in SCCP/0919/05, results from animal studies can be used to categorise skin sensitisers in three groups according to their sensitising potency: extreme, strong and moderate. The LLNA provides dose-response data that can be used to derive an EC3 value, which is the estimated concentration of a chemical necessary to give a 3-fold increase in lymph node cell proliferation compared to vehicle-treated controls (SI ≥ 3). This EC3 value is used to subcategorise skin sensitisers (**Table 7**) (Basketter *et al.*, 2005, ECB, 2002).

Table 7: Potency subcategorisation of skin sensitisers

Category	***EC3 value (%)***
Extreme	≤0.2
Strong	>0.2 - ≤ 2
Moderate	>2

Because the guinea pig test methods often do not provide dose-response data, the intradermal induction concentration in the GPMT and the topical induction concentration in the Buehler test are used for subcategorisation (Basketter et al., 2005, ECB, 2002). In the absence of LLNA data, this subcategorisation can be used as indicative for potency.

The **Skin Sensitisation Quantitative risk assessment (QRA)** has been developed for fragrance substances, only. The basic principles of the QRA are presented in SCCP/1153/08. It is based on the dose of a sensitising chemical, not expected to cause induction of sensitisation (No Expected Sensitising Induction Level (NESIL), which may be derived from animal and human data. The NESIL is adjusted by a number of uncertainty factors (Sensitisation Assessment Factors, SAFs) in order to calculate an acceptable exposure level (AEL). In addition, a consumer exposure level (CEL) is calculated. The AEL is then compared with the CEL, whereby, for an acceptable risk, the AEL should be greater than or equal to the CEL. Within the IDEA project (http://www.ideaproject.info) the QRA was further refined by including aggregate exposure assessment and revising the SAFs.

This revised QRA (QRA 2) has been evaluated by the SCCS (SCCS/1589/17) and it was concluded that a lot of progress had been achieved since the initial publication of the QRA. However, it is not yet possible to use the QRA2 to establish a concentration at which induction of sensitisation of a fragrance is unlikely to occur. Several aspects of the methodology are not clear and the scientific rationale behind the methodology needs to be better described. With some revision, this could be a useful methodology not only for safety evaluation of fragrance allergens, but potentially also for other cosmetic ingredients.

In particular, in the case of new substances, post-marketing surveillance would be essential (see also SCCS/1459/11) to monitor that their use in cosmetics does not lead to allergic contact dermatitis in consumers, in line with the SCCS Memorandum on use of human data (SCCS/1576/15).

3-4.8 REPEATED DOSE TOXICITY

Repeated dose toxicity studies are performed to investigate 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.

A. NAMs

No validated alternative method is available yet for determining the repeated dose toxicity of a substance, which poses a problem for new compounds as this assay usually provides the PoD of the compound under investigation (necessary for MoS calculation).

B. In vivo

The following ***in vivo* repeated dose toxicity studies** with OECD guidelines are available:

1)

- Sub-acute oral toxicity (28 days) (EC B.7, OECD 407)
- Sub-acute dermal toxicity study (28 days) (EC B.9, OECD 410)
- Sub-acute inhalation toxicity study (28 days) (EC B.8, OECD 412)

2)

- Sub-chronic oral toxicity study: repeated dose 90-day oral toxicity study in rodents (EC B.26, OECD 408)
- Sub-chronic oral toxicity study: 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 (primarily rodents) (EC B.30, OECD 452)
- Combined chronic toxicity/carcinogenicity studies (primarily rodents) (EC B.33,OECD 453)

In the case of the development of cosmetic ingredients that 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.

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 to consider systemic exposure.

The 28-day and 90-day oral toxicity tests in rodents have been the most commonly used repeated dose toxicity tests and often gave a good indication on the target organs and the type of systemic toxicity. Whenever available, studies for duration of 90 days or more should be used in safety assessments of cosmetic ingredients**.** If only a **28-day study** is 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 most of the lifespan of the animals. In these tests, effects which require a long latency period or which are cumulative may also manifest.

The inhalation route was only rarely used in repeated dose toxicity testing of cosmetic ingredients due to the lack of relevance for the majority of cosmetic products. This exposure route is, however, important where a cosmetic ingredient is volatile or a 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 3-3.4 and 3-3.5).

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 associated 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 (PoD) is then derived for each study. If the dose regimen of a study was limited to 5 days treatment per week, the derived PoD will be corrected by a factor of 5/7. In analogy, a correction will also be done for longer use periods. 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 evaluation (see Section 3-5.1).

3-4.9 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.

A. NAMs

No validated alternative method is yet available for reproductive toxicity that covers all different phases of the reproductive cycle (JRC 2016, 2017).

Since the field of reproductive toxicity is very complex, it is expected that the various phases 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 three following categories: non-embryotoxic, weak/moderate-embryotoxic or strong-embryotoxic. The WEC test is still an animal test and is considered scientifically valid only for identifying strong embryotoxic substances (ESAC, 2001).

These three 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 complex endpoint of reproduction toxicity is not covered by the above systems.

Several *in vitro* methodologies, each covering one of the three biological components of the reproductive cycle (male and female fertility, implantation and pre- and postnatal development), were developed under the EU project ReProTect.

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. Neverthless, more information and research are needed until regulatory acceptance can be envisaged (Schenk *et al.*, 2010).

An extensive review of the actual situation can be found in a JRC report (JRC, 2016, 2017). In view of the utmost importance of consumer safety, toxicological evaluation against some complex endpoints, such as reproductive toxicity, still necessitate the use of animals.

B. <u>In vivo</u>

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

1) Two-generation reproductive toxicity study (EC B.35, OECD 416)
2) Prenatal developmental toxicity study[3] - rodent and non-rodent (EC B.31, OECD 414)

There also exists a "Reproduction/Developmental Toxicity Screening Test" (OECD 421), as well as a "Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test" (OECD 422).

The Extended One-Generation Reproductive Toxicity Study (EOGRTS) has been adopted by the OECD (OECD 443) and a Guidance Document has been established (OECD 2013a). It offers several advantages compared to older OECD TGs and is extensively used:

- Compared to OECD TG 416 a significant number of animals can be saved.
- More parameters are addressed (*e.g.* clinical-chemical parameters as in repeated dose studies; developmental immunotoxicity and neurotoxicity in case such cohorts are included). Endocrine disruption endpoints are included- (*e.g.,* nipple retention, anogenital distance at birth, vaginal patency and balanopreputial 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 study report on reproductive toxicity or on prenatal developmental toxicity is in general only acceptable when it is based on tests that have been carried out before the animal testing ban or when generated for compliance with other (non-cosmetic) legislative frameworks; see Section 1 and **Appendix 4**).

3-4.10 MUTAGENICITY / GENOTOXICITY

Mutagenicity: a mutation means a permanent change in the amount or structure of the genetic material in a cell. The term 'mutation' applies both to heritable genetic changes that may be manifested at the phenotypic level and to the underlying DNA modifications when known (including specific base pair changes and chromosomal translocations). The term 'mutagenic' and 'mutagen' is used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms.

Genotoxicity: the more general terms 'genotoxic' and 'genotoxicity' apply to agents or effects which alter the structure, information content, or segregation of DNA, including those which cause DNA damage by interfering with normal replication processes, or which in a non-physiological manner (temporarily) alter its replication.
Germ cell mutations are those that occur in the egg or sperm cells (germ cells) and therefore can be passed on to the organism's offspring. Somatic mutations are those that occur in cells other than the germ cells, and they cannot be transmitted to the next generation (ECHA, 2017).

Based on recommendations of international groups of scientific experts (Dearfield *et al.*, 2011), and in consensus with EFSA (EFSA, 2011a) and the UK Committee on Mutagenicity (COM, 2011), the evaluation of the potential for mutagenicity of a cosmetic substance should include information on 1) mutagenicity at the gene level, 2) chromosome breakage and/or

[3] Often also named teratogenicity test

rearrangements (clastogenicity), and 3) numerical chromosome aberrations (aneuploidy). For this task genotoxicity tests, which measure irreversible mutation endpoints (gene or chromosome mutations), should be used. Indicator tests, which measure DNA damage without taking into account the consequences of this primary damage, can provide confirmatory evidence but 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.

A. NAMs

From a 3-test battery to a 2-test battery

Evaluation of several databases has demonstrated that an increase in the number of *in vitro* 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 and COM (2011) recommended the use of these 2 tests as a first step in genotoxicity testing. According to the REACH Regulation and ECHA Guidance (2017), in order to ensure the necessary minimum level of information is provided, at least one further test is required in addition to the gene mutation test in bacteria, namely: an *in vitro* chromosome aberration test (OECD TG 473), or an *in vitro* micronucleus test (OECD TG 487) using mammalian cells. Although *in vitro* chromosome aberration test is considered as a possible alternative option to the *in vitro* micronucleus test under REACH, it is now generally agreed that these tests are not equivalent since the *in vitro* chromosome aberration test is not optimal formeasuring numerical chromosome aberrations.

In line with this, 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.

The tests should be performed according to the OECD test guidelines.

Cells should be exposed to the test substance both in the presence and absence of an appropriate metabolic activation system. The most commonly used system is a cofactor supplemented S9-fraction prepared from the livers of rodents (usually rat) treated with enzyme-inducing agents such as Aroclor 1254 or a combination of phenobarbital and β-naphthoflavone. The choice and concentration of a metabolic activation system may depend on the class of chemical being tested. In some cases, it may be appropriate to utilise more than one activation system. For azo dyes and diazo compounds in the gene mutation test in bacteria, the use of a reductive metabolic activation system is recommended (SCCS/1532/14).

In cases where the bacterial reverse mutation test is not optimal for the measurement of nanoparticles, biocidal compounds and antibiotics, a scientific justification should be given and a gene mutation test in mammalian cells {a*Hprt* test (OECD 476), or a mouse lymphoma assay (OECD 490)} should be performed.

Additionally, when testing nanomaterials, evidence is needed to show that the nano-particles were in contact or internalized by the test system. For further considerations of particle-related behavior of substances, the Applicants should refer to SCCS/1484/12: Guidance on the Safety Assessment of Nanomaterials in Cosmetics (under revision).

Outcome of *in vitro* tests

- 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 is needed to exclude potential mutagenicity (and/or clastogenicity) of the substance under investigation.

A general scheme of mutagenicity testing of cosmetic ingredients is presented in **Figure 5.** Additional information on the *in vitro* testing can be found in COM2011.

Different and potentially contradicting results may be available from the same test, performed by different laboratories or on different occasions. In such a case, expert judgement should be used to evaluate and interpret the data. It may be necessary to carry out another test to reach an overall conclusion.

Special attention should be given for poorly soluble chemicals. For such substances that are not cytotoxic at concentrations lower than the lowest insoluble concentration, the highest concentration analysed in culture medium should produce turbidity or a precipitate visible by eye or with the aid of an inverted microscope at the end of the treatment with the test chemical. Even if cytotoxicity occurs above the lowest insoluble concentration, it is advisable to test at only one concentration producing turbidity or with a visible precipitate because inaccurate effects may result from the precipitate. At the concentration producing a precipitate, care should be taken to assure that the precipitate does not interfere with the conduct of the test (*e.g.* staining or scoring). The determination of solubility in the culture medium prior to the experiment may be useful.

Toolbox for further evaluation in a WoE approach

- The comet assay in mammalian cells or on 3D-reconstructed human skin is a tool which can support a WoE approach in the case of a positive or equivocal gene mutation test in bacteria or mammalian gene mutation test.
- To evaluate a positive or equivocal result, the *in vitro* micronucleus test on 3D-reconstructed human skin (RSMN) could be considered for dermally applied compounds. The experimental phase of the validation of the tests has been finalised (Aardema *et al.,* 2013; Roy *et al.,* 2016, JRC 2017).
- Another tool is the Hen's Egg test for Micronucleus Induction (HET-MN) which is currently under evaluation (JRC 2016, 2017).

Negative results from these alternative tests on their own might not be sufficient to overrule the positive results from a recommended test.

- Mechanistic investigations (*e.g.* toxicogenomics) or internal exposure (toxicokinetics) are tools that may be helpful in a WoE evaluation.

- Reporter gene assays based on human, animal or bacterial cells are tools supporting a WoE approach. Among such tests are the Green Screen HC™ used to screen the genotoxic and cytotoxic potential of chemicals and ToxTrackerTM, which when combined with Vitotox (a mutagenicity test that can be used as a surrogate for an Ames test) showed a better performance than observed in the official 2-test battery (Ates *et al.,* 2016).
- The information gained by a reporter assay provides mechanistic information on the molecular level but cannot alone overrule a positive result from an *in vitro* battery as it is based on a limited number of genes.
- Another tool to potentially address a positive result in a 2-test battery (in one of the two assays) is transcriptomics analysis in TK6 cells (Li *et al.,* 2015), HepG2 cells (Maghoufopoukou *et al.,* 2012) or HepaRG™ cells (Ates *et al.,* 2018), in which a higher number of genes provide mechanistic information.
- The determination of the level of phosphorylated form of H2AX histone (γH2AX) in cells exposed to a chemical can provide information on its potential for induction of the DNA double-strand breaks (DSB) (Georgoulis *et al.* 2017). Assays that simultaneously analyse different biomarkers (*e.g.,* p53, γH2AX, phospho-histone H3 or polyploidy) are being developed to provide mechanistic information on the types of biological damage induced by different classes of substances (Bryce *et al.*, 2017).

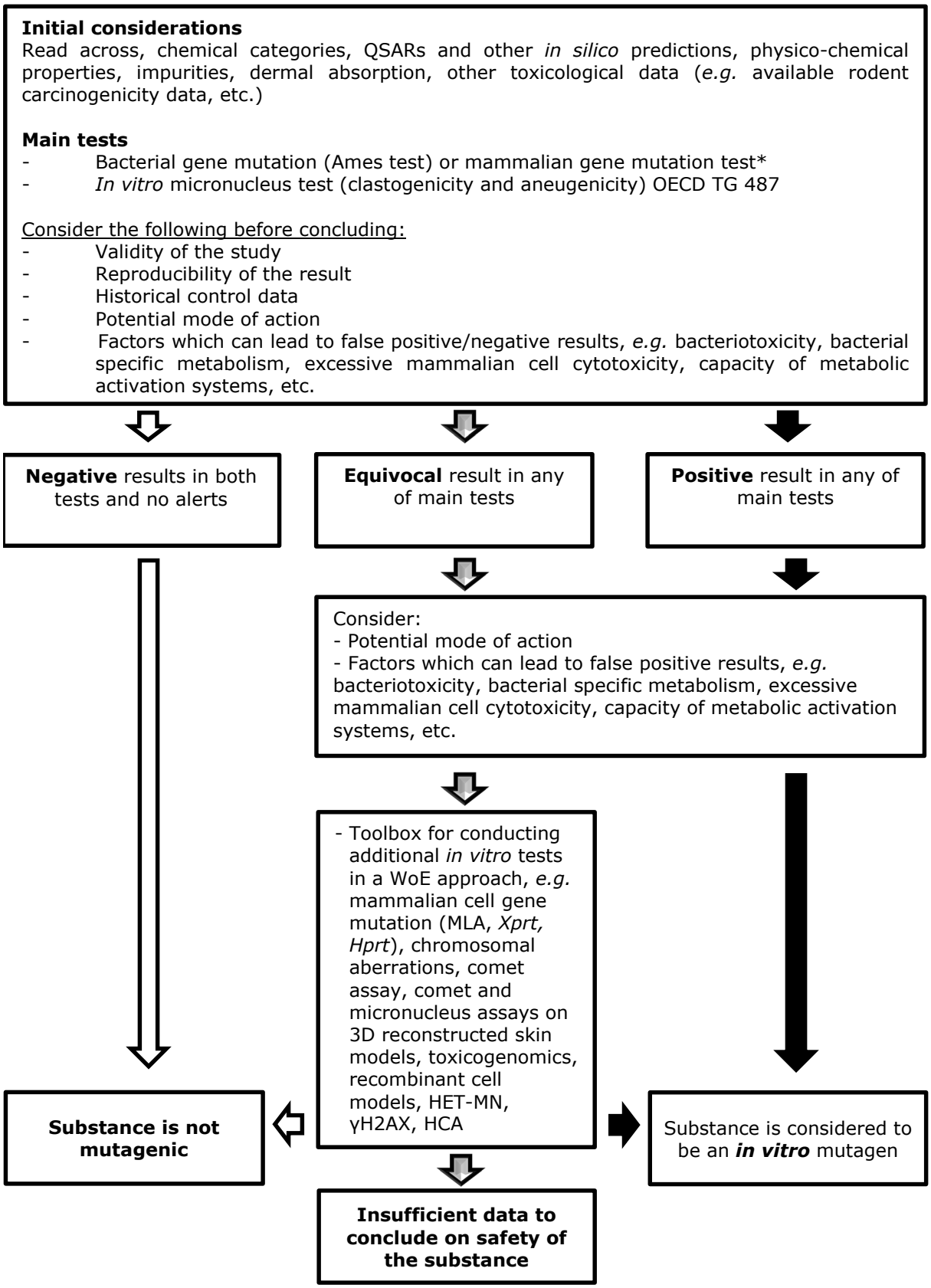
Despite the possibilities offered by the toolbox, expert judgement may be necessary to be able to come to a conclusion.

Intensive work is being carried out on adapting current tests to high-throughput technologies (*e.g.*, micronucleus, Comet assay, γH2AX, high content analysis and other assays; Collins *et al.,* 2017).

Alternative tests for which no OECD test guideline is currently available should be performed according to the general principles laid down in OECD test guidelines (OECD 211).

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

The SCCS has published an Addendum to the NoG (SCCS/1501/12), in which details such as definitions, critical steps, crucial experimental conditions to be followed, etc. are described (SCCS/1532/14).



* Bacterial gene mutation test is preferable. If not suitable (*e.g*. in the case of antibiotics, nanomaterials), mammalian gene mutation should be provided

Abbreviations: MLA – Mouse Lymphoma Assay; *Xprt* – Xanthine-guanine phosphoribosyl transferase gene; *Hprt* – Hypoxanthine-guanine phosphoribosyl transferase gene; HET-MN - hen's egg test for micronucleus induction; γH2AX – phosphorylated form of H2A histone family member X; HCA – High Content Analysis

Figure 5. Scheme of testing strategy for genotoxicity/mutagenicity of cosmetic ingredients

B. *In vivo*

When there is a positive result from an *in vitro* gene mutation test, adequate somatic cell *in vivo tests* are:

-a Transgenic Rodent and Germ Cell Gene Mutation Assay TGR (OECD TG 488),

-an *In vivo* Mammalian Alkaline Comet Assay (OECD TG 489).

It is no longer recommended to perform an Unscheduled DNA Synthesis (UDS) test with mammalian liver cells *in vivo* (OECD TG 486) (EFSA, 2017b).

Adequate somatic cell *in vivo* tests to investigate structural or numerical chromosome aberrations are:

-a Mammalian Erythrocyte Micronucleus Test (OECD TG 474),

-a Mammalian Bone Marrow Chromosome Aberration Test (OECD TG 475)

-an *In vivo* Alkaline Comet Assay (OECD TG 489).

EFSA concluded that target tissue exposure in *in vivo* studies should be demonstrated, particularly in the bone marrow (*e.g.*, Mammalian Erythrocyte Micronucleus assay). Toxicity to the bone marrow in itself provides sufficient evidence to allow concluding on the validity of a negative outcome of a study. All other direct or indirect evidences of target tissue exposure should be assessed within a weight-of-evidence approach.

Animal studies on mutagenicity or genotoxicity are acceptable when data are already available from tests that have been carried out before the animal testing ban or when generated for compliance with other legislative (non-cosmetic) frameworks (see Section 1).

3-4.11 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 (ECHA 2017).

Carcinogens are often differentiated as "genotoxic carcinogens" (DNA reactive substances) for which the most plausible mode of carcinogenic action includes the consequences of genotoxic effects (*i.e.* point mutation and structural chromosomal aberrations) and "non-genotoxic carcinogens" (NGC), or non-DNA reactive substances that are carcinogenic due to mechanisms other than direct interactions with DNA (ECHA, 2017).

A. NAMs

At present validated alternative *in vitro* methods as OECD test guidelines to determine the carcinogenic potential of substances are not available. However, there are new *in vitro* approaches which may be helpful in an overall WoE approach to indicate potential genotoxic as well as non-genotoxic carcinogenic (NGC) substances.

For genotoxic substances, *in vitro* mutagenicity tests are well developed. Due to the relation between mutations and cancer, these genotoxicity tests can also be seen as a pre-screening for carcinogenicity. A positive result in one of the *in vitro* mutagenicity/ genotoxicity testing battery may be indicative for considering a substance as a putative carcinogen. This indication may be further supported by a positive result in Cell Transformation Assays (CTAs, Guidance documents No 214 and No 231).

Worldwide research is ongoing with regard to *in vitro* toxicogenomics for the detection of mutagens, genotoxic carcinogens, and particularly NGC. By global gene expression profiling *via* microarray technology, gene patterns covering diverse mechanisms of substance-induced genotoxicity can be identified.

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; Ates *et al.* 2018). In addition to *in vitro* mutagenicity/genotoxicity tests (see above), data from *in vitro* tests combined with toxicogenomics may also be considered in a WoE approach.

Current regulatory requirements and problems with respect to non-genotoxic carcinogens

NGC either induce mutations in (short term) eukaryotic and prokaryotic mutation assays or induce direct DNA damage in the target organ. Although it has been estimated that 10-20% of recognised human carcinogens classified as Class 1 by IARC act through NGC mechanisms (Hernandez *et al.,* 2009), there are no specific requirements to obtain information on NGC mechanisms of carcinogenicity. As such many NGC will remain unidentified, and as a consequence their risks to human health will not be managed. The overview of NGC mechanisms presented by Jacobs *et al.* (Jacobs *et al*., 2016) indicates that assays with endpoints capturing early key event mechanisms may provide an individual contribution to the WoE approach of NGC.

Due to the limitations mentioned an integrated approach to testing and assessment (IATA) for NGC has been developed. As such it is possible to consider the CTAs as one of the possible building blocks of the IATA. All CTA models provide morphological endpoints of onco-transformation, which can be used as phenotypic anchoring for mechanistic studies (Callegaro *et al*., 2017). An experimental protocol which combined the BALB/c 3T3 CTA and a global gene expression analysis was developed to highlight the cross-talk between genotoxic and non-genotoxic carcinogenic mechanisms in the pathway leading to malignant cell transformation (Vaccari *et al.*, 2014). The toxicogenomics approach applied to the *in vitro* CTA allowed the identification of the transcriptionally activated pathways (Mascolo *et al.*, 2018). This integrated approach has the potential to be considered to be part of an IATA for non-genotoxic carcinogenesis (Corvi *et al.*, 2017).

Cell Transformation Assays (CTA) as a possible alternative to animal models

CTA can detect both genotoxic and NGC (Sasaki *et al*., 2014). They measure cell transformation, which is one step in the multistep cancer process. It addresses several endpoints (for more information, see **Appendix 8, Table A.8**). 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 WoE assessment (Doktorova *et al.,* 2012, Creton *et al.*, 2012). When employed in combination with other information, such as genotoxicity data, structure–activity analysis and pharmaco/toxicokinetic information, CTAs could facilitate a relatively comprehensive assessment of carcinogenic potential (Creton *et al.*, 2012, Corvi *et al.*, 2017, Mascolo *et al.,* 2018).

Two Guidance Documents on CTA, OECD No. 214 (OECD, 2015) and OECD No. 231 (OECD, 2016), can be used in a WoE approach in the testing of substances for carcinogenic potential. At present, the carcinogenic potential of a substance cannot be derived from a stand-alone CTA.

B. *In vivo*

Usually carcinogenic potential of a substance is assessed using a 2-year bioassay (OECD 451: Carcinogenicity Studies). A combined chronic toxicity/carcinogenicity study can also be performed to identify carcinogenic and the majority of chronic effects, and to determine dose-response relationships following prolonged and repeated exposure (OECD 453: Combined Chronic Toxicity/Carcinogenicity Studies).

An *in vivo* carcinogenicity study is only acceptable when based on tests that have been carried out before the animal testing ban or when carried out for the purpose of compliance with other (non-cosmetic) legislative frameworks (see Section 1).

3-4.12 PHOTO-INDUCED TOXICITY

3-4.12.1 PHOTO-IRRITATION AND PHOTO-SENSITISATION

A. NAMs

The "3T3 Neutral Red Uptake Photo-toxicity Test (3T3 NRU PT)" is a validated *in vitro* method (EC B.41, OECD 432), 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 ultraviolet/visible (UV)/VIS) light. Its use is mandatory for testing for phototoxic potential. 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.

A statement on photo-toxicity testing is available from UK COM (2013). This Committee recommends a revision of OECD 432, in order to require photo-toxicity assessment if the UV/VIS molar extinction/absorption coefficient of the active substance and its major metabolites is greater than 1000 L x mol^{-1} x cm^{-1} (instead of 10 as mentioned in Reg. (EU) No. 283/2013 and OECD 432). The experts agreed that further guidance is needed with regard to the UV/VIS molar extinction/absorption coefficient of the active substance for values between 10 and 1000 L x mol^{-1} x cm^{-1}.
EFSA (2016) concluded that for a light source emitting wavelengths mainly below 320 nm, more guidance is needed on how to interpret the data and on how to perform the test with a light source emitting between 290 and 320 nm. In the OECD TG, it is mentioned that cytotoxicity increases 1000-fold as the wavelength goes from 313 to 280 nm. Although the data requirement in Reg. (EU) No. 283/2013 are for substances absorbing electromagnetic radiation in the wavelength range 290-700 nm, there are difficulties in testing below 320 nm. The EFSA proposed that the photo-toxicity test should not be performed if it has been demonstrated that the test material only absorbs at wavelengths lower than 313 nm and if there is insufficient absorption at longer wavelengths.

As a second tier, the biological effects can be further evaluated on a reconstructed human skin model with some barrier properties (Kandarova, 2011). A positive control should always be included. A negative result for the compound under consideration is usually accepted. To enhance the chance of achieving correctly predicted results of photo-toxic potential of chemicals, a more complex screening using UV/VIS light spectral analysis and Reactive Oxygen Species (ROS)/micellar ROS (mROS) assays could be used according to Nishida *et al.* (2015).

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. There is also work conducted on some other *in vitro* tests for photo-allergenic potential such as: photo-h-CLAT, NCTC2455 assay, dendritic cell-based assay, or photo-SH/NH2 test (Onoue *et al.,* 2017).

B. *In vivo*

At present, no official guideline-based protocols for photo-irritation and photo-sensitisation 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 not, however, specify protocols for the testing of adverse effects of orally or topically applied agents, nor do they give recommendations about the species to be used.

3-4.12.2 PHOTO-MUTAGENICITY / PHOTO-GENOTOXICITY

Photo-mutagenic or photo-genotoxic chemicals are chemicals that absorb visible or UV light and, through activation to a more reactive state or release of free radicals, cause damage to DNA and induce gene mutations or chromosome aberrations.

The terms ''photomutagenesis'' or ''photogenotoxicity'' are used to describe the 'indirect' induction of gene mutations or chromosomal aberrations after transfer of energy or charge from a light absorbing molecule other than DNA (Muller and Gocke, 2013). This includes the genotoxic effects elicited by degradation products and/or radicals generated by light of VIS and UV wavelengths.

Current status of tests available for photo-genotoxicity/photo-mutagenicity assessment

A previous version of the Notes of Guidance (SCCNFP/0690/03) already mentioned that for the detection of photo-chemical clastogenicity/mutagenicity, several assays had been adapted to a combined treatment of chemicals with UV-VIS light (Averbeck *et al.*, 1979; Dean *et al.*, 1991; Chetelat *et al.*, 1993a,b, 1996; Gocke *et al.*, 1998; Pflaum *et al.*, 1998; Kersten *et al.*, 2002).
The existing principles and test methods in the field of photo-mutagenicity/photo-genotoxicity was summarised in the report of the Gesellschaft für Umweltmutationsforschung (GUM) Task Force on photochemical genotoxicity (Brendler-Schwaab *et al.,* 2004). 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. In many cases, the concurrent use of irradiation, while performing a standard mutagenicity/genotoxicity study, does not significantly alter the existing OECD protocol without irradiation. Therefore, the majority of the described photo-mutagenicity/photo-genotoxicity tests are considered as being valid.

In their comprehensive review, Müller and Gocke (Müller and Gocke, 2013) concluded that "photo-mutagenicity is not suitable for a general testing framework within cosmetic or pharmaceutical testing guidelines" and suggested a case-by-case approach.

Guidances for photogenotoxicity/photomutagenicity testing

The COM (COM 2013) recommended that photogenotoxicity testing does not need to be undertaken routinely as part of a photosafety assessment and that photogenotoxicity testing had a negligible impact in the overall assessment for potential of photocarcinogenicity. Moreover, if there is a negative response from the phototoxicity test, no photomutagenicity test is required. However, in the case the test is positive, no specific guidance was provided.

The International Conference on Harmonisation (ICH) guideline on photosafety evaluation of pharmaceuticals (Step 4 of the ICH Process dated 13 November 2013) stated: 'Note 2. Testing for photogenotoxicity is not recommended as a part of the standard photosafety testing program as in most cases, the mechanism by which compounds induce photogenotoxic effects is identical to those that produce phototoxicity, and thus separate testing of both endpoints is not warranted.'

The ICH guideline has been adopted in EU by the Committee for Medicinal Products (CHMP) in December 2015 and issued as EMA/CHMP/ICH/752211/2012 (EMA, 2015) as well as in USA by FDA and issued as FDA/2013/D/0068 (FDA, 2015).

In 2016 the EFSA (2016) agreed that photomutagenicity testing is not required for the time being, unless further guidance is provided. Additionally, they concluded that the concern regarding positive results in the phototoxicity test should be raised to the risk managers in the conclusion of the peer review.

In this regard, taking also into consideration the general recommendations regarding the experimental conduct of tests for photo-genotoxicity (Gocke *et al.,* 2000), is as follows:

- although the validity of photo-mutagenicity/photo-genotoxicity testing is being questioned, in specific cases when the structure of a molecule, its light absorbing potential or its potential to be photo-activated may indicate photo-mutagenic/photo-genotoxic hazard, then photo-mutagenicity tests should be provided, including gene mutations and clastogenicity/aneugenicity endpoints; especially when the substance is liable to reach the eyes or light-exposed areas of skin, either by direct contact or through systemic distribution. Additionally, available alternative methods, for example scientifically validated comet assay for detection of oxidized DNA lesions, or *in silico* methods can be considered.
- UV-VIS spectra of the compound along with Molar Extinction Coefficient (MEC) determined according to harmonized procedure should be provided.
- the photo-toxicity test should not be performed if the test material only absorbs at wavelengths lower than 313 nm and if there is insufficient absorption at longer wavelengths.
- no photo-mutagenicity tests are needed when the phototoxicity tests are negative.
- there is no requirement for photo-mutagenicity testing of compounds with a MEC below 1000 L mol^{-1} cm^{-1}.

3-4.13 HUMAN DATA IN HAZARD ASSESSMENT

Tests in animals and alternative methods may have limited predictive value with respect to the human situation. Therefore, when human data is available, this information is very valuable. Human data can be obtained *via* various sources. For bioavailability and systemic toxicology information, sources could be: post-marketing surveillance data, results from biomonitoring programs (see also Section 3-4.14), 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, tests with human volunteers.

Tests with human volunteers (*e.g.* skin compatibility tests) confirm that there are no harmful effects when applying a cosmetic product for the first time to human skin or mucous membranes. If considered scientifically and ethically necessary, human tests can only be envisaged, provided that the toxicological profiles of the components are available and no concern is raised. A high degree of safety needs to be ensured. Finished cosmetic products are usually tested in a small group of human volunteers to confirm skin and mucous membrane compatibility, as well as cosmetic acceptability (fulfilment of in-use expectations).

Human studies might also become necessary to build up and validate PBPK models (see Section 3-3.5.3).

The general ethical and practical aspects related to human volunteer compatibility studies on finished cosmetic products, are described in SCCNFP/0068/98 (for skin irritancy) and SCCNFP/0245/99 (for skin sensitisation). For skin sensitisation, human patch test data, if available, have to be taken into account (SCCS/1567/15).

Predictive testing of potentially skin sensitising cosmetic (mixtures of) substances (SCCNFP/0120/99) is more controversial than the irritancy testing, since these tests carry the risk of inducing a long-lasting or permanent immunological sensitisation in the individual. Therefore, serious ethical questions arise.

Despite many years of experience with human sensitisation tests, limited scientific information is available regarding the consequences involved for human volunteers who have developed sensitisation as a result of such testing.

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

The same ethical restrictions apply to human predictive tests on photosensitisation. For photosensitisation, information can be obtained from published clinical studies and case reports. There are no officially adopted guidelines or protocols, but in general the test procedures are quite similar to those 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.

3-4.14 HUMAN BIOMONITORING

In most risk assessment frameworks for chemicals, the default approach to calculate exposure is to assess intake from different sources and different routes of exposure. Different sources and routes are often assessed separately without aggregating exposure. This approach includes various uncertainties and depending on the scope of the assessment may over- or underestimate the real uptake. Overestimation may result from combining several conservative parameters in a deterministic assessment, whereas real life exposure may be underestimated by not taking into account all relevant sources.

Human Biomonitoring (HBM) is therefore an important tool to survey the real life internal exposure of humans resulting from 'total' exposure to chemicals *via* different routes (lung, skin, digestive tract). By providing more accurate data on actual internal exposure, inclusion of HBM data could improve human health risk assessment to consumer products for both the general population (exposure *via* air, consumer products, drinking water and food) as well as for workers (exposure *via* inhalation and/or skin), separately, or as part of the population (Santonen, 2018).

3-4.14.1 DEFINITION

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

For the assessment of non-adverse biological effects also the term "Effect-Monitoring" is used.

3-4.14.2 FIELDS OF APPLICATION

Besides the use of HBM for exposure assessment, population-based HBM has emerged to investigate the possible association between internal exposure to certain substances and human health status and trends of exposure.

For cosmetic ingredients, the risk of systemic effects is largely determined by skin absorption, which can be measured *in vitro* (OECD 428) (Section 3-3.5.1.1). 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 aggregate exposure, biomonitoring data may be useful to estimate the internal dose of exposure resulting from different sources and routes of exposure (CMRs, Section 3-6.6). Quantification by using biomarkers of exposure is increasingly used to provide an integrated

measure of a person's multiple chemical-specific exposures. Pharmacokinetics should also be taken into account (*e.g.* non-persistent, semi-volatile chemicals are metabolised quickly. HBM data as such are, however, not suitable for the assessment of a specific exposure to a (cosmetic) substance when other (non-cosmetic) sources for uptake and exposure also contribute considerably to exposure. HBM should rather be used as support in risk assessment and risk management. Back-calculation from biomonitoring data to external exposure data is possible but requires additional information (*e.g.,* type of biomarker, exposure modelling).

If adequately applied (*i.e.* toxicokinetics and metabolism of a substance are 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. 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 without the need for interspecies extrapolation, or the limitation of a small number of subjects involved in human volunteer studies. Ethical restraints usually do not pose a problem. If sufficient animal data are available, intraspecies variation can also be addressed using HBM.

3-4.14.3 OTHER CONSIDERATIONS

When HBM in used in the safety evaluation of consumer product ingredients, the following limitations apply:

- 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 the uptake of a substance from the environment or consumer products.
- HBM is not appropriate when the relevant biomarker is non-specific (*e.g.,* can be formed by different parent compounds such as hippuric acid).
- 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).

3-5 GENERAL PRINCIPLES FOR THE CALCULATION OF THE MARGIN OF SAFETY AND THRESHOLD OF TOXICOLOGICAL CONCERN

3-5.1 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 POD_{sys} (usually historical NOAEL or BMD values from oral studies) and an estimate of the exposure.

Mostly, only a repeated dose toxicity study with *oral* exposure is available as surrogate for a study with dermal exposure. For comparison with the POD_{sys}, usually an SED for the dermal route is derived as the exposure estimate. For calculation of SED, see 3-3.5.4.
Where possible, a BMD is used as POD_{sys} {see also 3-1 (3)}.

$$\mathbf{MoS} = \frac{\mathbf{POD_{sys}}}{\mathbf{SED}}$$

3-5.1.1 THE POD VALUE {SEE SECTION 3-1(3),(4)}

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 should be taken into account. The evaluation should include an assessment of the severity of the effect(s), whether the observed effect(s) are adverse or adaptive, irreversible or not -and whether they are precursors or not of significant effects 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 the effects. Further guidance on this issue can be found in several publications (WHO, 1994; WHO, 1999; ECETOC, 2002; ECHA, 2012a).

The 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**.

The BMD approach should preferentially be used as the dose descriptor for the Point of Departure (POD) and the MoS calculation (EFSA, 2009). When no BMD can be calculated, usually historical NOAEL values are applied.

If a BMD or a NOAEL cannot be identified from the available data, other dose descriptors such as the Lowest Observed (Adverse) Effect Level (LOAEL) may be used in the MoS calculation.

<u>Determination of BMD</u>

Although not limited to *in vivo* data, it involves first fitting a dose-response model to the data and then interpolating to find the dose that causes a predefined response. That dose is defined as the BMD. To account for uncertainty and provide a margin of safety, a two-sided 90% confidence interval for the BMD is calculated and the lower limit of that interval, the BMDL, is employed instead of the NOAEL to calculate the PoD. The upper limit of the BMD interval, the BMDU, is sometimes used to calculate the BMDU/BMDL ratio which provides an estimate of the uncertainty in the BMD value. The BMD/BMDL ratio can also be used for this purpose but is less good as it is does not take the full uncertainty in the BMD estimation into account (EFSA guidance, 2017c).

With quantal data, also referred to as dose-response data, the outcomes are incidences, *e.g.* number of animals with signs of toxicity. With such data the BMD is defined as the dose that gives rise to a Benchmark Response (BMR), most often defined as either an increased additional risk or extra risk. An extra risk of 10% is recommended as default for the BMR by both EFSA (EFSA, 2016) and US EPA (US EPA, 2010).

Body weight, organ weights and enzyme levels are typical continuous data, also referred to as dose-effect data. For such data each animal has its own magnitude of effect and the arithmetic or geometric means of the different dose groups are usually compared.

EFSA has proposed a preferred default 5% as a BMR, with modifications if required by toxicological or statistical considerations (EFSA, 2017c).

Choice of models

Application of different models to the same data will yield different values for the BMD and BMDL. As a consequence, there are different methods that guide the choice of which BMD and BMDL to use. Current EFSA guidelines suggest that the lowest BMDL among the models that pass a goodness-of-fit test should be used as the PoD (EFSA, 2017c). EPA´s guidelines are less conservative, suggesting that the model with the lowest Akaike Information Criterion (AIC) should be used as the PoD, unless there is a large difference between the BMDL values obtained with the different models (US EPA, 2012).

The AIC takes the likelihood of the model fit into account, but penalizes models with many parameters:

SCCS considers that there are still practical considerations regarding the use of this approach when evaluating cosmetic ingredients and its application requires a level of expert judgement and modelling expertise.

Adjustment factors to the POD

Dependent on dosing regimen, adjustment to daily exposure should be performed. For example, if the dose regimen in such a study was only 5 days treatment per week, a POD corrected by a factor of 5/7 should be used for the MoS calculation (ECHA, 2012a).

When the POD is based on a LOAEL, often an additional assessment **factor of 3** is added in the calculation of the MoS. 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 when LOAEL values are used). In some cases, the study cannot be used for safety assessment.

In case a 90-day repeated dose toxicity study is not available, a NOAEL or BMDL 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.

3-5.1.2. THE PODSYS VALUE

For most of the cosmetic ingredients evaluated by the SCCS, the SED is compared to an oral POD. Generally, the POD 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 usually calculated by dividing the internal (systemic) PODsys by the SED.

If the absorption by the oral route is 100%, then the external and internal doses of the oral route are the same. If the absorption by 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.

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 used** as the default oral absorption value for a cosmetic ingredient and the PODsys is derived from the POD by dividing with a factor 2. If there is information to suggest **poor oral bioavailability, a default value of 10% oral absorption could be considered**. However, whenever oral absorption data are available, these should be used, also when using other dose descriptors. Also, any other available kinetic data should be considered.

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.

In the case of **oral to inhalation extrapolation, a default factor of 2**[4] is also proposed (default absorption oral route: 50%; inhalation 100%; ECHA, 2014b).

3-5.1.3 MoS Analysis

The calculated MoS is compared with a reference MoS, which is comparable to the uncertainty/assessment factor used in risk and safety assessments to extrapolate from a group of test animals to an average human being, and subsequently from average humans to sensitive subpopulations (see **Figure 6**). A default value of 100 (10x10) accounting for inter- and intraspecies differences is generally accepted and a MoS of at least 100 therefore indicates that a cosmetic ingredient is considered safe for use.

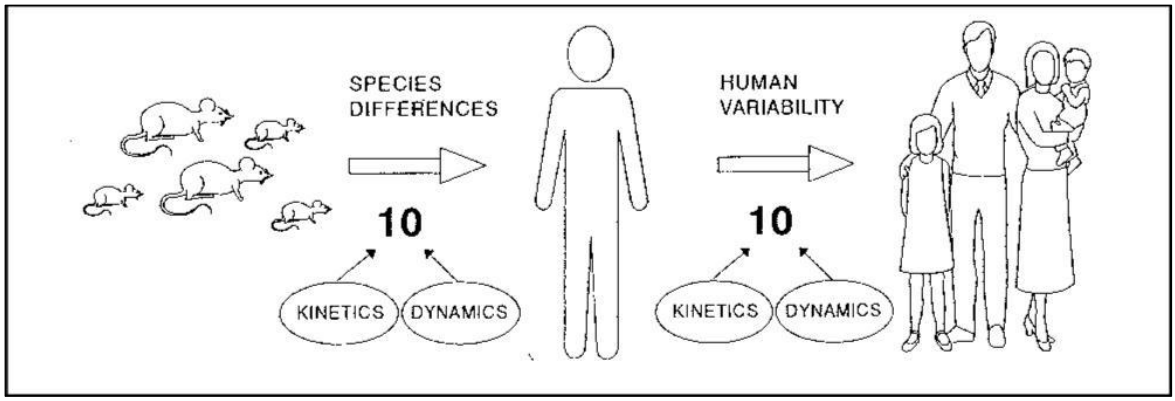
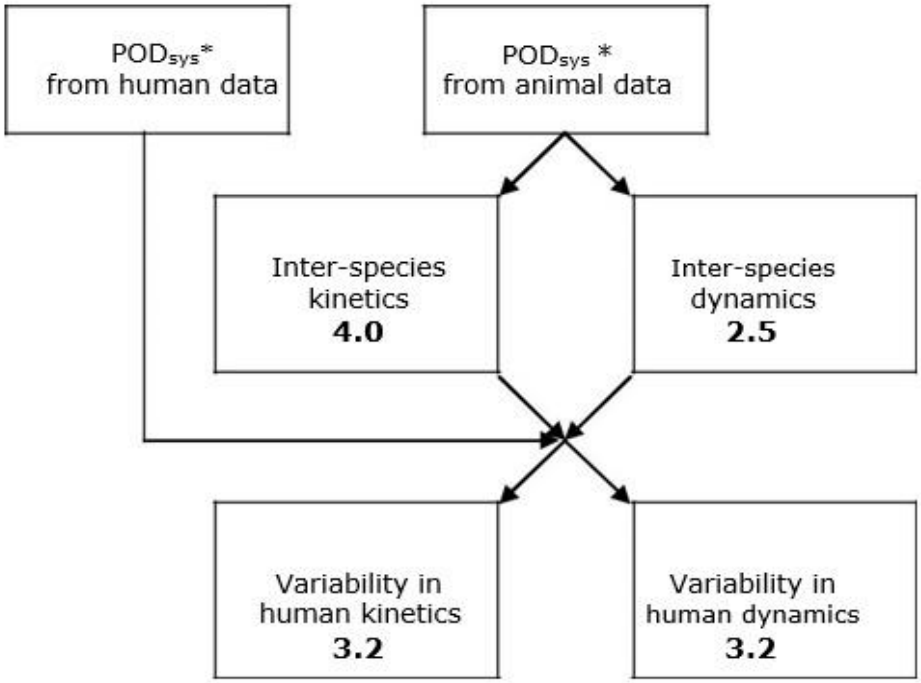


Figure 6: Schematic representation of the extrapolation from animal to man (Renwick, 1998).

As shown in **Figure 6**, the default value of 100 consists of a factor of 10 for the extrapolation from test animals to an average human being (interspecies extrapolation) and another factor of 10 taking into account the variations within the human population (intra-species extrapolation). These factors can be further subdivided as indicated in **Figure 7**.
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 intra-species toxicokinetic default factor (see **Figure 7**) can be increased (case-by-case evaluation).

Regarding substance-specific information for variations in toxicodynamics, deviation from the default value is possible if sufficiently justified. 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)

[4] Besides the default value of 50% for oral absorption, in this guidance, another default value of 50% for dermal absorption should be distinguished if no adequate dermal absorption data is available {see Section 3-3.5.1 (b)}.



* including historical NOAEL values

Figure 7: Further subdivision of the uncertainty/assessment factor, taking toxicokinetics and toxicodynamics into account (based on WHO, 1994).

Additional considerations:

i. Some cosmetic substances are not used on a daily basis, although their NOAEL values have been obtained in studies after daily administration of the substances. Combining these NOAEL values with daily exposures therefore results in 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 therefore accepted as a conservative estimate, even if it is only applied *e.g.* once per week or once per month. However, the daily amount for product categories with low frequencies of application may not be adjusted by the frequency (*i.e.* not divided by 30, if applied once per month), 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 (*e.g.* oxidative hair dyes only once applied per month) 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.

ii. When 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 3-6.10 and 3-5.2). See also for example UV filter HAA299 SCCS/1533/14.

iii. 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**.**

iv. 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 decimal digits in the final MoS is therefore not recommended.

3-5.2 THE THRESHOLD OF TOXICOLOGICAL CONCERN (TTC)

3-5.2.1 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 pragmatic risk assessment tool that is based on the principle of establishing human exposure threshold values for all chemicals below which there is a very low probability of an appreciable risk of systemic adverse effects to human health.

Use of the TTC concept for chemicals with specific data requirements for their regulatory approval under a specific European regulation, is currently not acceptable as an alternative to a chemical-specific evaluation.

Nevertheless, the TTC concept has been acknowledged to be a science-based prioritisation and risk assessment tool by different organisations such as WHO IPCS, EFSA, SCCS, SCHER, Health Canada (Joint FAO/WHO Expert Committee on Food Additives, 1996; EFSA, 2012; SCCS, SCHER, 2012; EFSA, 2016; SCCS NoG, 2016; Health Canada, 2016).

EFSA (EFSA, 2012) concluded that the TTC approach should not be used for the following (categories of) chemicals: high potency carcinogens (*i.e.* aflatoxin-like, azoxy- or N-nitroso-compounds, and also benzidines and hydrazines); inorganic chemicals; metals and organometallics; proteins; steroids; chemicals that are known or predicted to bioaccumulate; nanomaterials; radioactive chemicals and mixtures of chemicals containing unknown chemical structures.

So far, this approach has been used in a regulatory context for food contact materials (only in the USA), food flavourings, genotoxic impurities in pharmaceuticals, genotoxic constituents in herbal preparations and for pesticide metabolites in groundwater.

The TTC approach, aims to screen and prioritise chemical compounds for which the chemical structure and exposure data are known, but no or limited toxicity data is available, using an algorithm developed by Cramer (Cramer, 1978) where the substances, depending upon their chemical structure are grouped into three structural classes (low, medium, high safety concern) in comparison with the toxicity data from available databases.
A database containing carcinogenicity data from animal studies for more than 3500 carcinogenicity experiments (Carcinogen Potency Database) (Gold *et al.,* 1984) and a database containing 613 chemicals based on toxicity other than carcinogenicity (Munro database) (Munro *et al.*, 1996) were available when the TTC approach was developed. Both are based on systemic effects after oral exposure.

As with any risk assessment tool, application of the TTC approach 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.

3-5.2.2 TTC APPROACH FOR HUMAN HEALTH RISK ASSESSMENT OF CHEMICAL SUBSTANCES AND COSMETIC SUBSTANCES

The Scientific Committees (SCs) consider the TTC approach, in principle, scientifically acceptable for human health risk assessment of systemic toxic effects caused by chemicals present at very low levels of exposure. The application of the TTC should, however, be done on a case-by-case basis and requires expert judgement. The TTC approach is also 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 small number of misclassifications of compounds when using the Cramer decision tree in its present form have been revealed.

Misclassification may also result in a classification to a higher toxicity class. (Bhatia *et al*, 2015; Yang *et al*, 2017) and hence still be conservative for safety evaluation.

The SCs concluded that the TTC value of Cramer Class II is not supported by the available databases and these substances should be treated as Class III substances. The SCs also accepted in principle the division of substances into Cramer Classes I or III (EFSA, 2016a). When assigning a chemical to the lowest toxicity **Class I, 1800 µg/person/d corresponding to 30 µg/kg bw/d the** classification should be carefully considered and justified. If classification in Class I cannot be justified, the SCs recommended a general default value equivalent **to Class III compounds, being 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 (Boobis *et al.,* 2017).

The SCCS agreed that, **the default value of 0.15 µg/person/d, corresponding to 0.025 µg/kg bw/d** can be used **for chemicals with genotoxicity alerts** and hence possible DNA reactive carcinogens but recommends its scientific basis to 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 BMD_5 or BMD_{10} as PoD 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 (*i.e.* 30 µg/kg bw/d for Class I, 1.5 µg/kg bw/d for Class III, 0.0025 µg/kg bw/d for chemicals with structural alert for genotoxicity) 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.

In the EU SEURAT-1 project COSMOS, work has been done on the TTC substances with non-genotoxic alerts that are used for cosmetic purposes. The COSMOS TTC dataset, which is quality controlled, contains 552 chemicals (495 cosmetic ingredients) with 219, 40, and 293 chemicals in Cramer Classes I, II, and III, respectively, to expand the chemical space and to provide more robust thresholds for cosmetic-related chemicals. A TTC of **7.8 mg/kg-bw per day is suggested for Cramer Class III** (which is 5-fold higher than the corresponding TTC value was derived by Munro *et al.,* 1996). **Cramer Class II was insufficient** for derivation of a robust TTC value. For **Cramer Class I, a moderately increased TTC of 49 mg/kg-bw per day** is proposed, (Yang *et al*, 2017).
It is important to note that the TTC values derived from the COSMOS dataset have not yet been evaluated by the SCCS.

It is also noteworthy that an appropriate exposure assessment is essential for the application of the TTC approach.

For cosmetics, the main exposure route is dermal. In the proposal from Kroes *et al.* (2007), an external exposure value was converted to an internal exposure value by use of an adjustment factor for percutaneous absorption. The latter value was then compared to the TTC value as if the TTC value is also an internal exposure value. This is the case under the assumption of 100% oral bioavailability, which in many cases is an overestimate. For proper route-to-route extrapolation, the NOAELs from the Munro database need to be corrected for oral absorption. It should be mentioned that in only few cases quantitative information on absorption after oral administration is available.

For cosmetic ingredients any risk assessment as well as the TTC approach should be based on internal doses (Partosch *et al.*, 2014). Therefore, when the TTC approach is applied for cosmetic ingredients, an adjusted internal TTC value has to be defined considering both dermal and oral absorption. Further work in this area is currently ongoing.

3-6 SPECIAL CONSIDERATION FOR CERTAIN COSMETIC INGREDIENTS

3-6.1 MULTI-CONSTITUENT NATURAL INGREDIENTS

Many cosmetic ingredients can be mixtures of multiple substances of natural origin, *e.g.* essential oils and fragrances; they often can 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:

- qualitative identification and 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 mixtures of variable composition, an indication of the range and the maximum levels of components which may be present in the mixture, taking into account batch to batch variation;
- a clear indication of the cosmetic product category in which the mixture may be used and at what maximum concentration.

In the final safety evaluation, reference should be made to the semi-quantitative composition of the multi-constituent ingredient. The toxic potential of each component should be considered individually and the mixture 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).

3-6.2 IDENTIFICATION OF MINERAL, ANIMAL, BOTANICAL AND BIOTECHNOLOGICAL INGREDIENTS IN A COSMETIC PRODUCT

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

a) Complex substances of mineral origin

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

b) Complex substances of animal origin

When animal-derived cosmetic substances are used, this should be clearly mentioned (see 3.6.3)

- 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, proteins, 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, known 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 microorganism 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 microorganisms
- host pathogenicity
- toxicity, and when possible, identity of metabolites, toxins produced by the organisms
- fate of viable organisms in the environment-survival-potential for transfer of characteristics to *e.g.* natural bacteria
- physical and chemical specifications
- microbiological quality
- additional external contamination
- preservatives and/or other additives added.

3-6.3 ANIMAL-DERIVED COSMETIC SUBSTANCES

When animal derived cosmetic substances are used, this should be clearly mentioned.

Entry no. 419 in Annex II of Reg. 1223/2009/EU specifies a number of substances for which some concern exists for human health with respect to transmissible spongiform encephalopathy (TSE).

"**419.** *Category 1 material and Category 2 material as defined in Articles 8 and 9, respectively of Regulation (EC) No 1069/2009 of the European Parliament and of the Council of 21 October 2009 and substances derived therefrom*[5]."

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

Finally, taking into account EC Regulation No 1069/2009 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 (SCCP/0933/05). Category 3 material is not intended for human consumption, but it may be used as cosmetic substance in accordance with Regulation 1069/2009, Article 33.
Non-animal derived supplements for *in vitro* testing should be used wherever possible. The chemically defined/serum-free cell culture media can be found in several *in vitro* test methods for skin corrosion, skin irritation and eye irritation testing (OECD 431, 439 and 492) (van der Valk *et al.* 2017).

3-6.4 SUN PROTECTION SUBSTANCES

For **sunscreen lotion**, an amount of **18.0 g/day** is used in the MoS calculation. It is used as a standard exposure value in the safety evaluation carried out by the SCCS **but is not meant as a recommended amount to be applied by the consumer** (SCCNFP/0321/02). To reach a comparable level as indicated by the sun protection factor (SPF), sunscreen products have to be applied in quantities similar to the ones used for SPF testing, *i.e.* 2 mg/cm^2 (total amount of approx. 36 grams) for the body of an average adult person (2006/647/EC). The quantity of 2 mg/cm^2, however, is the amount necessary to obtain reproducible SPF results under laboratory conditions. It is higher than the amount usually applied by consumers.

[5] OJ L 300, 14.11.2009, p. 1

This observation has been reported frequently: when consumers use their own sun products (lotions, alcoholic solutions, gels, creams, sprays,…) and apply the products on the whole body surface, values for use of products between 0.5 - 1.3 mg/cm² have been reported (Stenberg *et al.*, 1985; Bech-Thomsen *et al.*, 1993; Diffey, 1996; Gottlieb *et al.*, 1997; Autier *et al.*, 2001 and 2007). The values seem to be depending on the study protocol used, the location on the body measured and several other factors. More recent publications still come to comparable values in the range of 0.39-1 mg/cm² (Danish Protection Agency No. 151, 2016, Ficheux *et al.,* 2016a, Gomez-Berrada *et al.,* 2017). When the product is applied only to the face, then the amount applied might be higher than 2 mg/cm^2 (Gomez-Berrada *et al.,* 2017). The amount used by the SCCS in safety calculations reflects actual consumer use and takes the whole body area (17500 cm^2) into account. The average exposed skin area of sunscreen users according to the recent report of the Danish authorities is 14,700 cm^2.

The use of 18g/d sunscreen corresponds with the values reported by Biesterbos *et al.* (Biesterbos *et al.,* 2013), who found a mean use amount of 9.2 g/application, derived on the basis of pictures. If two applications are considered this is about 18 g/d. Unpublished data by von Goetz (von Goetz, 2018) from a small-scale pilot study with weighing also provided a mean of 9 g for whole-body application (5 applications by 2 persons).

If a sun protection substance is applied in a sprayable product that may give consumer lung exposure by inhalation, other considerations should be taken into account (see3-3.4.1.3). For lipcare products, 100% absorption of the substance should be considered for safety assessment.

3-6.5 ENDOCRINE ACTIVE SUBSTANCES (EAS)

3-6.5.1 DEFINITIONS

Some natural and synthetic chemical substances can interact, interfere or disrupt the function of the endocrine system that regulates various metabolic and developmental functions in the body (WHO/IPCS, 2002; UNEP/WHO, 2012). The endocrine system comprises a complex array of signalling and feedback mechanisms, the disruption of which has been linked to various adverse health effects, such as obesity, diabetes, cancers, reproductive effects, and immunological and metabolic disorders. However, the endocrine system also involves numerous cycles and feedback loop mechanisms and adaptive responses that together regulate the secretion of hormones and maintain homeostasis. A substance interfering with the endocrine system may affect hormone secretion or other cellular factors, but it is possible that such perturbations remain within the homeostatic or metabolic detoxification capacity and therefore do not result in adverse effects in the intact organism. Some effects linked to endocrine disruption have also been shown to have critical window(s) of susceptibility, *e.g.* increased susceptibility of an organism within a certain developmental period.

A number of chemicals have been identified, or are suspected, as endocrine disruptors (EDs). However, "only a small fraction of these chemicals has been investigated in tests capable of identifying overt endocrine effects in intact organisms" (WHO-UNEP report, 2012).

The joint EFSA/ECHA/JRC draft guidance (EFSA and ECHA, JRC, 2018) has defined endocrine activity as 'Interaction with the endocrine system which can potentially result in an effect on the endocrine system, target organs and tissues'.

The definition of EDs endorsed at the European level[6] is the same as proposed by WHO/IPCS (WHO/IPCS, 2002) as follows:

"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".

[6] Commission Regulation (EU) 2018/605 of 19 April 2018 amending Annex II to Regulation (EC) No 1107/2009 by setting out scientific criteria for the determination of endocrine disrupting properties. OJ L 101, 20.4.2018, p. 33–36.

The OECD's revised conceptual framework (OECD TG 150) also has a prerequisite to identify the adverse effect in an intact organism for regarding a substance an endocrine disruptor. Thus, whilst a chemical may be regarded an EAS on the basis of activity/interaction towards one or more components of the endocrine system (*e.g.,* a hormone receptor), it can only be regarded as an ED if there is evidence for a biologically-plausible causal relationship between the endocrine perturbation/activity and the adverse effect(s) in an intact organism (EFSA, 2013).

3-6.5.2 STEPWISE APPROACH FOR COSMETICS AND THEIR INGREDIENTS

Due to the animal testing ban under the Cosmetic Regulation, it is now out of scope to test cosmetic ingredients *in vivo* for endocrine disruption. Cosmetic ingredients therefore can be assessed for endocrine activity in a stepwise approach using data generated outside the cosmetic field or for a new cosmetic ingredient, using NAMs (*in silico* models, read across, *in vitro* assays, other mechanistic techniques such as 'omics'). Such characterisation will however be limited to the study of endocrine activity at level 1 (existing data and using *in vivo* data if they have been generated before the animal ban or for another regulatory purpose than cosmetics) and level 2 (*in vitro* assays) of the OECD's revised Conceptual Framework as described below:

- ***Lines of evidence level-1 (existing data and non-test information):***

The first level of evidence for endocrine activity of a substance may be provided by: physical and chemical properties (*e.g.,* MW, reactivity, volatility, biodegradability), all available (eco)toxicological data from standardised or non-standardised tests, read-across, chemical categories, QSARs and other *in silico* predictions, and ADME model predictions for a new compound intended for use in a cosmetic product, the use of *in silico* models and read-across tools, together with physicochemical data.

A number of *in silico* models and tools are available for the estimation of a substance's potential for binding with hormone receptors, such as estrogen receptor (ER), androgen receptor (AR), and pregnane X receptor (PXR). These include commercial programmes such as ADMET Predictor™ and MetaDrug™, as well as publicly available tools such as VEGA and Online Chemical Modeling Environment (OCHEM). Another open source docking tool Endocrine Disruptome is also available for virtual screening of EDs (see EFSA and ECHA, JRC, 2018).

In addition, a number of databases are available and provide some information on endocrine properties of chemical substances[7]. Criticism remains possible (*e.g.,* inaccurate information, some entries not enough documented). Endocrine Disruptor Screening Program (ESDP) Tier 1 screening assay results and the dataset from Collaborative Estrogen Receptor Activity Prediction Project (CERAPP) are also reported in (Mansouri *et al.*, 2016). These databases may also enable read-across for endocrine activity and provide a basis for further development of structure-activity based predictive models. Some of these databases also contain *in vivo* experimental data.

Amongst the available *in silico* tools, the OECD QSAR Toolbox offers a major software platform that incorporates several databases comprising chemical data, experimental (eco)toxicological data, and estimated values from QSAR tools, together with incorporated QSAR modelling tools and Expert Systems. For example, it contains:

- The OASIS Estrogen Binding Database, consisting of diverse compounds with relative Endocrine Receptor Binding Assay (ERBA) data. The Toolbox allows *in silico* screening of

[7] Endocrine active substances information system (EASIS) (EC JRC); ToxCast (US EPA); ToxCast ER prediction model (US EPA); SIN List (International chemical secretariat); The endocrine disruption exchange (TEDX); Endocrine disruptor screening program, EDSP21 (US EPA); Endocrine disruptor knowledge base, EDKB database (US FDA); Estrogenic activity database, EADB (US FDA); Toxicology data network (Toxnet); Developmental and Reproductive Toxicology database (DART); NURSA (nuclear receptor signalling atlas); OECD (Q)SAR toolbox (OECD, ECHA); AOP knowledge base (OECD); ToxRefDB (US EPA); eChem portal (OECD); COSMOS database - cosmetic ingredients; Danish (Q)SAR Database; (Q)SAR Data Bank

a compounds' endocrine activity through Danish EPA's Relative ERBA (Q)SAR, which is based on ER binding *in vitro*.

- QSAR models, including MultiCASE RBA QSAR, which is based on a hierarchical statistical analysis of a training set composed of ER binding data on a variety of chemical structures that are inactive, weak, or powerful ER binders.
- Structural-alert based ER-binding profiler to classify chemicals as non-binders or binders (weak, moderate, strong and very strong binders) depending on their MW and structural characteristics.
- Structural-alert based expert systems, such as the US EPA's rtER expert system based on binding to rainbow trout estrogen receptor.

The OECD QSAR Toolbox also provides a major platform for read-across between chemicals that share structural and/or functional similarities, using a substantial set of high quality databases. If compounds in the database are identified with the required structural and alert profile similarities to the target compound, they may be used as read-across candidates for the prediction of the ER binding of the target compound.
Other *in silico* systems based on molecular docking tools and 3D-(Q)SAR models are also available that allow virtual screening of chemical substances for affinity/binding with hormone receptors (Jacobs, 2004; Vedani *et al.,* 2012; Galli, 2014). The identification of affinity/binding to a hormone receptor by virtual screening, however, needs to be seen in the context of the scoring function used for each target, because a universally applicable scoring function is not yet available (Vuorinen *et al.,* 2013). Also, whilst *in silico* models can reliably predict simple endpoints, such as the binding free energy toward the receptor binding, they have a limitation for the prediction of more complex endocrine related *in vivo* endpoints, such as reproductive and developmental toxicity.

The available experimental data are still too scarce to allow comparison between the success rates of the results from different *in silico* methods (Vuorinen *et al.,* 2013).

- **Lines of evidence level-2 (*in vitro* assays providing data about selected endocrine mechanism(s)/ pathways(s) (mammalian and non-mammalian methods).**

Among the various endocrine modalities, Estrogen (E), Androgen (A), Thyroid (T) and Steroidogenic (S) - (EATS) modalities are the best characterised pathways. Other endocrine pathways, such as retinoid signalling or hypothalamo-pituitary-thyroid axis, are poorly investigated (Kortenkamp *et al.,* 2011; UNEP/WHO, 2012).

The currently available *in vitro* methods include estrogen, androgen, or steroidogenic receptor binding assays, whilst methods relevant to thyroid hormone are not sufficiently sensitive to completely exclude effects due to disruption of thyroid-related functions. A validation study on 17 methods for the detection of thyroid disruptors was launched by EURL ECVAM (JRC 2017). The available *in vitro* methods are listed below:

- Estrogen (OECD TG 493) or androgen receptor binding affinity (US EPA TG OPPTS 890.1150) (OPPTS stands for Test guidelines for pesticides and toxic substances).
- Estrogen receptor transactivation (OECD TG 455),
- Yeast estrogen screen (ISO 19040-1,2&3)
- Androgen receptor transcriptional activation (OECD TG 458)
- Steroidogenesis *in vitro* (OECD TG 456)
- Aromatase Assay (US EPA TG OPPTS 890.1200)
- Thyroid disruption assays (*e.g.*, thyroperoxidase inhibition, transthyretin binding)
- Retinoid receptor transactivation assays
- Other hormone receptors assays as appropriate
- High-Throughput Screens (See OECD GD No. 211 Describing Non-Guideline *In vitro* Test Methods: OECD 2014c)

Cosmetic ingredients suspected to have ED properties

As yet there is no harmonised approach towards health risk assessment procedures for EDs within the different regulatory frameworks in the EU. The SCCS has issued a memorandum (SCCS/1544/14) to clarify its position on substances with potential ED properties when used as cosmetic ingredients. In the context of the animal testing ban, it is not possible for the SCCS to fulfill the criteria as laid out under the OECD Conceptual Framework for identification of EDs for cosmetic ingredients in the context of the animal testing ban.

In the SCCS view, these substances should be treated like other substances of concern for human health and therefore be subject to risk assessment and not only hazard assessment. This is in agreement with the past and current evaluations by the SCCS in regard to the safety assessment of cosmetic ingredients with suspected ED properties *e.g.*, 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), cyclomethicone (SCCS/1241/10), decamethylcyclopentasiloxane (cyclopentasiloxane) (SCCS/1549/15).

At present, there is no official list of known or presumed EDs. However, some chemicals are being identified in REACH as Substances of Very High Concern (SVHCs) due to ED properties either for Human Health (*e.g.,* bisphenol A and phthalates such as BBP, DBP, DEHP and DiBP), or for the environment (*e.g.,* 4-tert-octylphenol and its ethoxylates, 4-nonylphenol and its ethoxylates).

Whilst the results from Level 1 and 2 approaches can be indicative of endocrine activity of a cosmetic ingredient, they will not definitively inform whether the substance will cause adverse effect(s) in the intact organism to be regarded an ED. In view of this limitation, it is important that all the evidence from physicochemical properties, available literature*, in silico* models, read-across, *in vitro* assays, and other techniques (such as "-omics") is integrated in a systematic manner to generate sufficient weight of evidence (WoE) to exclude the potential toxicity of a cosmetic ingredient through the endocrine related effects. Recently, the integration of *in silico* methods and computational systems biology has been proposed as a means to more critically assess the endocrine activity of chemical substances (Ruiz *et al.,* 2017).

Another way forward could be to demonstrate 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* evidence by providing data on (internal) exposure in relation to concentrations that were found to be active in *in vitro* assays (Coecke *et al*., 2013; Bessems *et al.,* 2014).

It also needs to be highlighted that the SCCS only assesses cosmetic ingredients in relation to safety of consumers' health, and as such they are not assessed for effects on the environment. Data generated on the environmental effects may, however, be also useful to support EA/ED mode of action but not their potency. For example, some ecotox tests may be informative for the assessment of endocrine activity of a compound in humans (*e.g.* Amphibian Metamorphosis Assay (AMA), Larval Amphibian Growth and Development Assay (LAGDA) or the thyroid effects).

3-6.6 CMR SUBSTANCES

The chemical legislation classifies substances that are *carcinogenic, germ and somatic 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. These substances could be allowed to be used as cosmetic substances within Europe under specific conditions, *e.g.* polyaminopropyl biguanide (PHMB – SCCS/1581/16).

CMR Cat. 1A or 1B substances may be used in cosmetics exceptionally where (1) they comply with the European food safety requirements[8], (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). An example for a CMR 1B substance is formaldehyde in nail hardener (SCCS/1538/14).

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 clarification and as 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.

3-6.7 NANOMATERIALS

3-6.7.1 DEFINITION OF NANOMATERIAL

Regulation (EC) No 1223/2009 specifically covers the use of nanomaterials in cosmetic products. The Regulation provides a definition of nanomaterial, as well as a mechanism for notification, labelling, and safety evaluation of cosmetic products containing nanomaterials. Under Article 2 (1) (k), "*nanomaterial" means 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".*

The Regulation therefore covers mainly those nanomaterials that are intentionally produced and are insoluble/poorly-soluble or biopersistent (*e.g.,* metals, metal oxides, carbon materials, etc.), and not those that are either completely soluble or degraded and are not persistent in biological systems (*e.g.,* liposomes, oil/water emulsions, etc.).

3-6.7.2 POTENTIAL SAFETY ISSUES OF NANOMATERIALS

The use of nanomaterials in cosmetics is subject to a high level of protection of human health under the EU Cosmetics Regulation. This is because nano forms of some substances may differ from their conventional (bulk) forms in terms of physicochemical properties, biokinetic behaviour, and/or biological effects. Any intended use of nanomaterials (other than colourants, preservatives and UV filters and not otherwise restricted by the EU Cosmetics Regulation) in cosmetic products must be notified to the Commission by the RP through the Cosmetic Product Notification Portal (CPNP) at least six months prior to placing them on the

[8] Regulation (EC) No. 178/2002

market, except where they had already been placed on the market before 11 January 2013. In case of a safety concern over a nanomaterial, the Commission shall request the SCCS for a scientific Opinion on the safety of the nanomaterial for use in relevant categories of cosmetic products in consideration of the reasonably foreseeable consumer exposure.

Whilst this section only provides a brief guidance on nanomaterials in cosmetics, the SCCS has published a more detailed specific Guidance on Risk Assessment of Nanomaterials (SCCS/1484/12) that is now being revised, 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), and a checklist for the applicants submitting dossiers on nanomaterials as cosmetic ingredients (SCCS/1588/17).

Safety assessors need to consult these documents to ensure that any testing to generate evidence on the safety of nanomaterials is carried out with special considerations of the nano-size related characteristics of the materials, and in compliance with the ban on animal testing of cosmetic ingredients. In this regard, it is important to note that, as indicated in the memorandum (SCCS/1524/13, Revision of 27 March 2014), the SCCS will only consider data that are relevant to the nanomaterial(s) under evaluation, are sufficiently complete, and are of appropriate quality to support the safety assessment.

The SCCS has also published a number of scientific opinions in the past few years on the nano-form of different materials. These include 1,3,5-triazine, 2,4,6-tris[1,1'-biphenyl]-4-yl-(ETH50) (SCCS/1429/11, revision of 13/14 December 2011); zinc oxide (SCCS/1489/12 revision of 11 December 2012); titanium dioxide (SCCS/1516/13, revision of 22 April 2014); carbon black (SCCS/1515/13, revision of 15 December 2015), 2,2'-methylene-bis-(6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol), MBBT (SCCS/1460/11), silica (SCCS/1545/15), hydroxyapatite (SCCS/1566/15); additional coatings for titanium dioxide (SCCS/1580/16); and titanium dioxide in sprays (SCCS/1583/17). These opinions can provide further information on the type of scientific evidence needed in a safety dossier on nanomaterials intended for use as cosmetic ingredients.

In general, a number of reviews have concluded that the existing risk assessment paradigm, in use for conventional chemicals, should in principle be also 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; SCCS, 2012; EC, 2012; ECHA, 2017; EFSA, 2018).

<u>Special features of nanomaterials:</u>

i. Due to high surface energies, nanoparticles have a tendency to stick together to form agglomerates and aggregates, and/or bind with other moieties on the particle surface. This particle behaviour can change in the presence of certain stabilising/dispersing agents. Characterisation of nanomaterials, prior to and during a test, is therefore a key to ensuring that results obtained are valid.

ii. Most of the currently available test methods were developed for conventional substances that can be solubilised. In contrast, nanomaterials generally comprise insoluble or poorly-soluble nanoparticles that are dispersed in a test medium in the form of a nano-suspension rather than a solution. The applied concentration of a nanomaterial may therefore drop during the test due to particle agglomeration, sedimentation, binding with other moieties in the medium, or sticking to the sides of the glass/plastic ware. This could lead to only a partial or no exposure of the test systems during the test. Nanomaterials are known to adsorb or bind different substances on their surfaces, including proteins (Šimon and Joner, 2008; Lynch and Dawson, 2008; Monopoli *et al.*, 2012; Moore *et al.*, 2015). They may also bind other substances in the test medium and carry them into the exposed test systems, leading to artefacts in the results.

iii. The toxicological hazards of chemical substances are currently measured and expressed in terms of weight or volume units (such as mg/kg, or mg/l). These conventional metrics may not be fully adequate to account for nanomaterial toxicity. It is therefore important that tests on nanomaterials are not only evaluated in terms of weight/volume concentration, but that results are also expressed in other dose-describing metrics, such as, particle number concentration, surface area etc.

iv. Due to the insoluble particulate nature, and the nano-dimensions, nanomaterials may show an altered uptake and biokinetic profile in a biological system compared to equivalent conventional forms *e.g.* transport of insoluble particles across biological membrane barriers is not driven by concentration-gradient based diffusion partitioning, but by other mechanisms such as endocytosis and/or active (energy-driven) uptake and transport.

v. Currently, there are uncertainties in regard to whether the endpoints identified by the current testing methods will be sufficient to identify and characterise all the hazards that may be associated with a nanomaterial.

3-6.7.3 REQUIRED INFORMATION FOR NANOMATERIALS

The information required by SCCS for the evaluation of nanomaterials as cosmetic ingredients is described in SCCS/1588/17 and SCCS/1484/12 (under revision).

The following aspects deserve special attention:

i. 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 for nanomaterials. The use of more than one method generally adds more confidence to the measured values *e.g.* for the measurement of particle size distribution, additional imaging by electron microscopy, has been recommended by both SCCS (SCCS/1484/12) and EFSA (EFSA, 2011b; EFSA, 2018).

ii. Where there is evidence for systemic absorption, further investigations are required to confirm whether the absorbed material was in a nanoparticle form or in solubilised/ionic/metabolised form. Where the absorption of nanoparticles cannot be ruled out either by experimental measurements 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 nano form.

iii. Surface modification/surface coating may bring about profound changes in a nanomaterial in regard to certain physicochemical properties and potentially the toxic effects.

iv. Therefore, a full dataset would be preferable. As a minimum, in addition to safety data on the core nanomaterial, the SCCS would require the following:

- Information/data on each material used for surface modification/coating of the nanomaterial to indicate that it is safe for use in the intended cosmetic product.
- Data on physicochemical properties of the surface-modified/coated nanomaterial to show that they have not significantly changed compared to either the same material when uncoated, or with a different surface modification/coating that has already been assessed safe by the SCCS.
- Data on dermal penetration, stability of the surface modification/coating, and (photo)catalytic activity, where relevant.

3-6.8 HAIR DYES AND HAIR DYE COMPONENTS

In April 2003 the Commission, together with the Member States, agreed on a step-by-step 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 SCCNFP (SCCNFP/0807/04) 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 the cosmetic legislation. Differentiation was made 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, semi-permanent and temporary 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).

3-6.8.1 MoS CALCULATIONS FOR HAIR DYE FORMULATIONS

Intermittent exposure and MoS calculations

Hair dyes are not intended to be applied on a daily basis. However, the MoS is calculated by dividing the PoD for daily application by the SED for a single application. Although this approach can be debated, this is used as a conservative approach.
Thus, the daily dose should not be averaged over the whole year (ECHA, 2012a).

3-6.8.2 ASSESSMENT OF OXIDATIVE HAIR DYE SUBSTANCES AND REACTION PRODUCTS

The SCCS is focused on the overall consumer health risk caused by ingredients as well as products and intermediates of oxidative hair dyes formed during hair dyeing processes (including their potential mutagenic/genotoxic/carcinogenic properties). The following conclusions are 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 these reaction products is considered generally lower compared to that from precursors and couplers since dimers and trimers are formed with higher molecular weight. No exposure to intermediates or self-coupling products was detected under experimental conditions. Therefore, in the risk assessment of reaction products toxicity is not considered a concern due to the low and intermittent exposure (on average once per month).
- The dermal 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).
- As no data have been made available for this endpoint, the sensitisation risk of the reaction products is not specifically addressed.
- The use of (Q)SAR in the case of reaction products is of limited value so far since the arylamine structure, a structural element of many hair dye precursors and reaction products, is automatically identified as an alert. 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.
- Although for precursors, couplers and reaction products positive results are commonly observed in *in vitro* genotoxicity assays there is no clear evidence of genotoxicity *in vivo* (in case *in vivo* data are available). It is possible that genotoxic effects can only be found at concentrations where the N-acetylation (detoxifying) capacity of the cells is overwhelmed, indicating that a 'first-pass' effect in skin could be taken into account for risk assessment of the topically applied aromatic amines (Zeller and Pfuhler, 2014; Nohynek *et al.,* 2015).
- The structures of the primary intermediates and trimer molecules reveal that they contain an aromatic secondary amino group, which if exposed to a nitrosating agent may form an N-nitroso derivative (Lewis *et al.,* 2013). Although, such transformation is theoretically possible, no evidence has been provided yet under real exposure conditions.
- For all the above reasons, the SCCS performs the safety assessment of oxidative hair dyes based on the toxicological evaluation of the ingredients (*i.e.* precursors and couplers) and not the reaction products.

With regard to the animal testing ban for cosmetic ingredients, see Section 3-1 and the scheme in **Appendix 4.**

3-6.9 COSMETIC INGREDIENTS FOR BABY AND CHILDREN PRODUCTS

Under certain circumstances it might be necessary to calculate the MoS for certain subpopulations such as children (*e.g.,* in case of exposure to 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 may be raised whether a higher MoS (above 100) would be required in order to cover children exposed to the ingredient seen in the light that there could be differences in metabolism between newborns/infants up to six months and adults.

3-6.9.1 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
- crawler/toddler 6 months – 2 years
- child/pre-adolescent 2 – 12 years
- adolescent 12 – 18 years

3-6.9.2 AGE-RELATED SUSCEPTIBILITIES/SENSITIVITIES

The calculation of the MoS for children was discussed when the question was raised 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 bodyweight values available at that time were used. Afterwards, updated values became available (EFSA, 2012).

The ratio between the SSA/BW ratios of children and adults changes from 0 to 10 years and is as follows (Renwick, 1998):

2.3 at birth,
1.8 at 6 months,
1.6 at 12 months,
1.5 at 5 years,
1.3 at 10 years.

These data indicate that the ratio between the SSA/BW children of 0 to 1 year of age and that of adults is at maximum 2.3. A factor of 3.2 is generally applied by the WHO covering also variability in human kinetics (see Section 3-5.1). Consequently, the inter-individual variation in SSA/BW is covered by the generally accepted default value of 100 for intact skin (**Figure 7** in Section 3-5.1). However, for specific compounds under consideration the potential differences in metabolism between newborns/infants up to six months and adults also require extra consideration. In general, there is no need for an additional uncertainty factor for children when intact skin is considered (SCCNFP/0557/02).
This point of view is taken by the SCCS.

Risk assessment in the specific case of "children" has been discussed in the use of parabens as preservatives in cosmetic products (SCCS/1446/11).

The rationale of an additional assessment factor for the different age groups beyond the usual factor of 100 has also 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 2 and 4 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. Potential risk factors for baby care products and their ingredients are described by Desmedt *et al.* (Desmedt *et al*., 2014).

Dermal exposure of the newborn and early infant[9]

- 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 and 2015).
- 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 intra-species 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, Felter *et al*, 2015).

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 generally be lower than that in 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 National Institute for Public Health and the Environment, the Netherlands (RIVM), ConsExpo Fact Sheet (2006).
Exposure data for wipes used for Korean babies are available (Lee *et al.,* 2017); also for USA, DE and UK as well deterministic as probabilistic modelling has been carried

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

out to determine the transfer of wipes in babies and children (Dey *et al.,* 2016a). Data for disposable diapers are available from the same authors (Dey *et al.,* 2016b).
CoE is preparing aggregate exposure data for babies and children for different baby care cosmetics used in Europe (more information, see **Table A.7, Appendix 7**).

(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 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 microorganisms:** 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.

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 demonstrates 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. *In silico* modeling of skin under the diaper has shown that healthy diapering practices will ensure there is no significant impact on skin health and barrier properties (Staadatmand *et al*., 2017). 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 safety evaluation of products intended to be used in the nappy area, the potential impact of irritation on dermal absorption of the ingredients needs to be considered by the safety assessor. It is known that the physico-chemical properties of the substances under consideration also play a role.

A tiered quantitative approach to take the potential for diaper rash into consideration when doing a safety evaluation for products used in the nappy area, has been proposed by Felter *et al.* (Felter *et al*., 2017).

3-6.10 SUBSTANCES WITH VERY LOW DERMAL ABSORPTION

In the case where a cosmetic ingredient is a substance with a very low dermal absorption {see Section 3-3.5.1.1(c)}, some studies could 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
- Local toxicity
- Mutagenicity/Genotoxicity
- High quality *in vitro* dermal absorption study, according to the SCCS Basic Criteria {3-3.5.1.1 (b}.

In these cases, the experimental mean value will be used for decision making.

3-7 FURTHER REMARKS FOR APPLICANTS

- When preparing a safety dossier, it would be useful if Applicants follow the same format as adopted in the SCCS opinions (example given in Appendix 3).
- 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.
- For multi-constituent natural ingredients, with variable composition, it is essential that the applicants provide clearly defined specifications in view of the range of variability of the components *e.g.* batch-to-batch.
- 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.
- The Applicant should ensure that files submitted for evaluation are complete and signed.

  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 (Adaptation to Technical and scientific Progress) Regulations, as well as the OECD test guidelines, and complying with the principles of Good Laboratory Practice (GLP). 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 by industry and/or relevant agencies.

- Together with the relevant experimental investigations, the following information should be provided:
  - for *in vivo* studies: the study date (whether in line with the Cosmetic Regulation) and/or the regulatory context for which the study has been performed;
  - any report on epidemiological and/or observational experiences (cosmetovigilance data);
  - an appraisal of all relevant published literature, along with a description of the bibliographical methods used; any information from "grey material" available. Any other relevant findings by the Applicant and/or other industry/agencies, should also be transmitted to the Commission for review.

- In their dossiers, the Applicants should indicate whether they consider any of the data/tables/substances names, etc. confidential (typically impurities etc.) for commercial reasons and provide relevant codes that can be used by the SCCS to anonymise the confidential information.
- Safety data must relate to the same form of ingredients as present in a product for final use keeping in view that the formulation or preparation of the final product may change the nature of the ingredients (*e.g.* permanent hair dye preparation).
- In case there is a negative SCCS Opinion, the Applicant must consider whether sufficient new and relevant information is available to justify a resubmission. When a dossier is resubmitted, it is mandatory to provide it in the form of a full dossier (including references) and clearly indicate what is new compared to the previous submission(s).

4. REFERENCE LIST

Regulations and Decisions from the Commission are ordered by year.

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

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

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

96/335/EC - Commission Decision of 8 May 1996 establishing an inventory and a common nomenclature of ingredients employed in cosmetic products. *Official Journal L 132, 01/06/1996 p.1.*

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

2004/210/EC - Commission Decision of 3 March 2004 setting up Scientific Committees in the field of consumer safety, public health and the environment *Official Journal L 66, 04/03/2004 p.45.*

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2006/647/EC- Commission Recommendation of 22 September 2006 on the efficacy of sunscreen products and the claims made relating thereto (notified under document number C(2006) 4089, OJ L 265, 26.9.2006, p. 39–43

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2009/146/EC - 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.*

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APPENDIX 1: INFORMATION ON REGULATION (EC) No 1223/2009 AND THE SCCS

1. INTRODUCTION TO COSMETIC REGULATION (EC) No 1223/2009

Since July 2013, Regulation (EC) No 1223/2009 harmonises the safety of cosmetics within the Member States, simplifies procedures and streamlines terminology. The most significant changes introduced by the 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" (RP)**
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 Cosmetics Regulation allows the precise identification of the RP and clearly outlines his/her obligations.

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

(4) **Introduction of reporting serious undesirable effects (SUE)**
A RP and a distributor have the obligation to notify serious undesirable effects to national authorities. The authorities will also collect information coming from end users and health professionals. 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**

(6) **A set of requirements for CMR** (carcinogenic, mutagenic, toxic for reproduction) **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.*

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.

2. THE SCIENTIFIC COMMITTEE ON CONSUMER SAFETY, SCCS

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, the Scientific Committee on Cosmetic Products and Non-Food Products intended for consumers (**SCCNFP**), was established. It was composed of independent scientists from different fields of competence, collectively covering the widest possible range of expertise.

In 2004, the SCCNFP was replaced by the Scientific Committee on Consumer Products (**SCCP**), as part of a larger-scale reorganisation of the EU Scientific Committees in the field of consumer safety, public health and the environment.

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 done by the Inter-Committee Coordination Group (ICCG).

In 2008, the three above-mentioned Scientific Committees were renewed[10] and the SCCP's name was changed into SCCS. 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 2013, the three above-mentioned Scientific Committees were renewed.[11]

Finally, a new Commission Decision C (2015)5383[12] was adopted on 7 August 2015, establishing two scientific committees: the (SCCS); the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER). The composition of both Committees was renewed on April 2016, for a period of 5 years until March 2021.

2-2 Mandate

The mission of the Scientific Committees is defined in Commission Decision C(2015)5383[13], 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; and toys, textiles, clothing, etc.

[10] 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
[11] 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
[12] http://ec.europa.eu/health/scientific_committees/docs/call_2015_5383_decision_with_annexes_en.pdf
[13] https://ec.europa.eu/health/sites/health/files/scientific_committees/docs/call_2015_5383_decision_with_annexes_en.pdf

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

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 (annex III)

(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

[14] See Article 31 of Regulation (EC) No 1223/2009

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 C(2015)5383[15] of 7 August 2015.

2-3 Rules of procedure

The Rules of Procedure[16] of the SCCS and SCHEER were jointly adopted by the Scientific Committees on 28 April products. These were amended according to the Commission Decision C(2015)5383.

In order to efficiently fulfil its extensive mandate, the SCCS 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[17] or *via* a specific call[18]). Working groups, for example, deal with: Cosmetic Substances (individual substance evaluations), 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 (or by written procedure) and published[19].
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 preliminary opinions, once edited, are published on the Commission's website[20] for a commenting period of a minimum of eight weeks to allow the applicant, and other stakeholders as well, to send their comments that are subsequently considered by the SCCS and, when considered appropriate, incorporated in a revised version of the opinion. The revised version becomes the final opinion once adopted at the next SCCS plenary meeting (or by written procedure) and is published on the website[21], with the date of the adoption of the final text. The final opinion replaces the preliminary opinion and informs about changes made in the first pages. The final opinions are not subject to further comments or revision requests. SCCS is not responding to comment submitted outside the commenting period. Any new data should be submitted directly to the responsible Commission unit mandating the SCCS for a new opinion.

This method of working with Working Groups 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.

[15] https://ec.europa.eu/health/sites/health/files/scientific_committees/docs/call_2015_5383_decision_with_annexes_en.pdf

[16] https://ec.europa.eu/health/sites/health/files/scientific_committees/docs/rules_procedure_2016_en.pdf

[17] http://ec.europa.eu/health/scientific_committees/experts/database/index_en.htm

[18] http://ec.europa.eu/health/scientific_committees/open_consultation/index_en.htm

[19] https://ec.europa.eu/health/scientific_committees/consumer_safety/requests_en

[20] https://ec.europa.eu/health/scientific_committees/consumer_safety/opinions_en#fragment0

[21] https://ec.europa.eu/health/scientific_committees/consumer_safety/opinions_en#fragment2

2-4 Opinions

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[22]. 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.

3. COMPLYING WITH THE TESTING AND MARKETING BANS

The safety evaluation of cosmetic ingredients is exposure-driven and is historically based on toxicological data, which were obtained by using experimental animals. The testing and marketing bans in Regulation (EC) No 1223/2009 make the use of validated alternative replacement methods compulsory. Guidance on how to comply can be found in:

i. the 50th recital of the Regulation,
ii. Commission Communication (COM/2013/135),
iii. a factsheet of ECHA (2014a) and
iv. the 2017 ECHA report (ECHA 2017) on the use of alternatives to testing on animals.

i. The 50th recital of Regulation (EC) No 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."*

ii. 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)"*

iii. A factsheet has 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 4**. It has to be noted that animal testing under REACH is not restricted, 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[24]).

[22] https://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04_en
[23] https://echa.europa.eu/documents/10162/13628/reach_cosmetics_factsheet_en.pdf
[24] "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.

iv. Additional information regarding the REACH legislation in the context of alternative methods can be found in the three reports on "The Use of Alternatives to Testing on Animals for the REACH Regulation", in the 3rd report under Article 117(3), available online (https://echa.europa.eu/documents/10162/13639/alternatives_test_animals_2017_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 question of the interpretation of the animal testing ban as regards animal testing performed in third countries to comply with the cosmetics legislation of a third country was referred to the European Court of Justice in case C-592/14. The Court concluded that: *''Article 18(1)(b) of Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products must be interpreted as meaning that it may prohibit the placing on the European Union market of cosmetic products containing some ingredients that have been tested on animals outside the European Union, in order to market cosmetic products in third countries, if the resulting data is used to prove the safety of those products for the purposes of placing them on the EU market*''.

The information provided in the NoG relates to the assessment of cosmetic ingredients from a general chemical safety point 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 (refer to SCCS/1484/12: Guidance on the Safety Assessment of Nanomaterials in Cosmetics (under revision).

APPENDIX 2: LISTS OF SUBSTANCES

1. INTRODUCTION

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

Another important list of cosmetic substances is the **INCI** (International Nomenclature Cosmetic Ingredient) inventory (96/335/EC) or CIN (2009/1223/EC), identifying a large number of substances with their possible function(s) in finished cosmetic products and with the nomenclature that needs to be used on the label of finished cosmetic products. DG 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. **The information contained in CosIng is indicative and does not have any legal value.**

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 3-6.6). With the 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 3-1.

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.

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 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 26.000 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 August 2018

APPENDIX 3: STANDARD FORMAT OF THE OPINIONS

Scientific Committee on Consumer Safety

SCCS

OPINION ON

.....

The SCCS adopted this document
at its plenary meeting/by written procedure on xx

ACKNOWLEDGMENTS

Members of the Working Group are acknowledged for their valuable contribution to this Opinion. The members of the Working Group are:

The SCCS members:

……

……
……
……

……

The SCHEER members (if applicable):

……
……

……

External experts (if applicable):

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……

The additional contribution of the following experts is gratefully acknowledged (if applicable):

XXXXXX

All Declarations of Working Group members are available on the following webpage:
http://ec.europa.eu/health/scientific_committees/experts/declarations/sccs_en.htm

If relevant: This Opinion has been subject to a commenting period of XXX weeks (from …… to ……) after its initial publication.

There were comments received and the final version of the Opinion includes information on XXXX (section concerned)….compared to the preliminary one.
There were changes/no change in the conclusions.

OR - There were no comments received and the final version of the opinion remained unchanged compared to the preliminary one.

1. ABSTRACT

The SCCS concludes the following:

Q1
Response
Q2
Response
Q3
Response
etc

Keywords: SCCS, scientific opinion, INCI name, type of product, Regulation 1223/2009, CAS ……, EC ……

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on INCI name - Submission …., preliminary version of (date), final version of (date), SCCS/…../XX……

About the Scientific Committees

Two independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

These Committees are: the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) and they are made up of scientists appointed in their personal capacity.

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.).

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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

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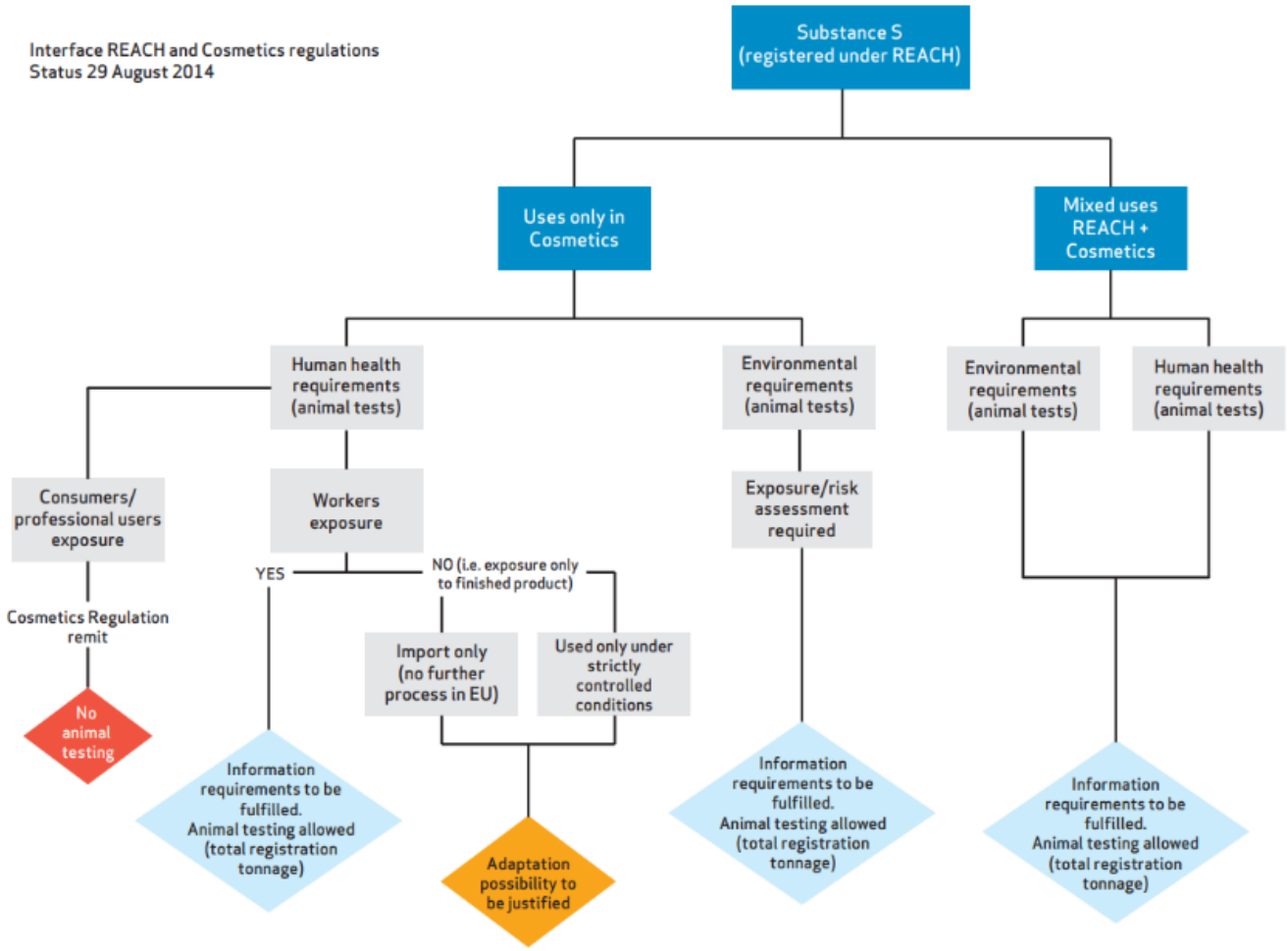
In alphabetical order

APPENDIX 4: ANIMAL TESTING: INTERFACE BETWEEN REACH AND COSMETICS REGULATIONS



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

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[25] (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[26]. 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.

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[27];
(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 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.

[25] OJ L 342, 22.12.2009, p. 59.
[26] OJ L 353, 31.12.2008, p. 1.
[27] OJ L 31, 1.2.2002, p. 1.

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 to allow that any possible derogation measure is adopted by the Commission within the timeframe defined by Article 15 of the Cosmetics Regulation 1223/2009.

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 Standing Committee on Cosmetic Products.

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[28]) 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[29] and sets up the conditions under which exchange of confidential data from REACH dossiers can be ensured with ECHA.

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

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 following the adoption of the classification as CMR substance.

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.

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)[30].

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 for the testing of cosmetic substances and their safety evaluation"[31].

[30] OJ L 396, 30.12.2006, p. 1.
[31] SCCS/1564/15 of 29 September 2015, revised on 16 March 2016.

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.

APPENDIX 6: REQUIREMENTS FOR THE CERTIFICATE OF ANALYSIS FOR A COSMETIC INGREDIENT

The Certificate of Analysis for a cosmetic ingredient should include:

1. The name and address of the laboratory performing the tests.
2. The registration number of the certificate of analysis.
3. The name, description and number of the batch for which the certificate is issued, the date of manufacture, and the expiry date.
4. The date on which the batch for which the certificate is issued was received.
5. A reference to the test procedure used, including the acceptance criteria (limits).
6. The results of all tests performed on the batch for which the certificate is issued (in numerical form, where applicable) and a comparison with the established acceptance criteria (limits), including information on Appearance, Identity (IR, NMR, MS), Purity, Solubility, Impurities (% content), Heavy metals.
7. Any additional test results obtained on samples from the batch as part of a periodic statistically based testing program
8. A statement indicating whether the results were found to comply with the requirements.
9. The date(s) on which the test(s) was (were) performed.
10. The signature of the head of the laboratory or an authorized person.
11. The name, address, and telephone and fax numbers of the original manufacturer. If supplied by repackers or traders, the certificate should show the name, address, and telephone and fax numbers of the repacker/trader and a reference to the original manufacturer.
12. A statement of the expected conditions of shipping, packaging, storage and distribution, deviation from which would invalidate the certificate.
13. A copy of the certificate generated by the original manufacturer, if the sample is supplied by a repacker or trader.

APPENDIX 7: DETAILED EXPOSURE DATA FOR COSMETIC PRODUCTS

During the last years exposure data for several cosmetic product categories became available in the open literature. These can be useful for safety assessors and safety agencies when in some particular cases refinement of risk assessment is necessary to show product or ingredients safety. In **Table A.7** a literature overview is provided of recent cosmetic product consumer exposure data (*e.g.* different categories of cosmetics with frequency of use, amount per application, amount per day) which are focused on consumers from one or more particular countries. In a number of cases, data are shown stratified by age and/or gender, and for different cosmetic formulations.

Table A.7: literature overview (2016-2018) of specific cosmetic consumer exposure data and assessments

Authors- year	***Country(ies)***	***Product categories***	***Additional information***
Gomez-Berrada *et al.* 2018a	France	tooth paste	adults and children; both genders
Gomez-Berrada *et al.* 2018b	France	sunscreens	adults and children; both genders under real-life conditions
Bernard *et al.* 2018	France	face and oral care cosmetic products	probabilistic exposure assessment; both genders; different age groups
Gomez-Berrada *et al.* 2017	France/ (1 city: Rennes)	cosmetic products	children under 2 years consumption; exposure assessment
Ficheux and Roudot 2017	France	cosmetic products	general population; both genders; different age groups
Dornic *et al.* 2017a	France	perfumes in cosmetic products	adults and children
Dornic *et al.* 2017b	France		default values for skin surface area
Dornic *et al.* 2017c	France	cosmetic products	exposure data; both genders, different age groups
Lee *et al.* 2017	Korea	baby care products	children 0-3 years
Garcia-Hidalgo *et al.* 2017	Swiss	personal care products	use patterns both genders; different age groups
Rieder *et al.* 2017		cosmetic ingredient	case of tea tree oil
Strittholt et a.l 2016		tooth paste	in children (2-7yrs)
Bernard *et al.* 2016a	France	hair dye products	both genders; use patterns; exposure assessment

Ficheux *et al.* 2016a	France	different cosmetic products	children (0-3yrs)
Ficheux *et al.* 2016b	France	different hair cosmetic products	both genders
Ficheux *et al.* 2016c	France	different cosmetic products	consumption amounts; different age groups; both genders
Dey *et al.* 2016a	USA, Germany, UK	baby wipes	lotion transfer *via* baby wipes
Dey *et al.* 2016b	world		exposure factor of disposable diapers
Comiskey *et al.* 2015	EU, USA	fragrance ingredients	probabilistic aggregate exposure
Manová *et al.* 2015	Swiss, Germany	UV filter ethylhexylmethoxycinnamate	probabilistic aggregate exposure
Tozer *et al.* 2015	USA	Zn pyrithione in rinse-off personal cleansing products	probabilistic aggregate exposure to
Dudzina *et al.* 2015		siloxane D5	probabilistic aggregate exposure (PACEM)
Nijkamp *et al.* 2015		fragrance geraniol in personal care products	probabilistic aggregate exposure
Safford *et al.* 2015		fragrance ingredients in cosmetic and personal care products	probabilistic aggregate exposure

APPENDIX 8: KEY CHARACTERISTICS OF CARCINOGENS

In the overall WoE assessment of a cosmetic ingredient 10 key characteristics commonly exhibited by established human carcinogens can be taken into account (Smith et al., 2016). High-throughput assay systems, such as the US EPA's ToxCast program (Chiu et al., 2017), which provide *in vitro* mechanistic data on several of the key characteristics, may be helpful.

Table A.8: Key characteristics of carcinogens (based on: Smith et al., 2016)

Characteristic	***Examples of relevant evidence***
1. Electrophilic or metabolically activated	Parent compound or metabolite with an electrophilic structure (*e.g.*, epoxide, quinone), formation of DNA and protein adducts
2. Genotoxic	DNA damage (DNA strand breaks, DNA–protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (*e.g.*, chromosome aberrations, micronuclei)
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (*e.g.*, topoisomerase II, base-excision or double-strand break repair)
4. Induces epigenetic alterations	DNA methylation, histone modification, microRNA expression
5. Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (*e.g.*, DNA, lipids)
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor in/activation (*e.g.*, ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
9. Causes immortalization	Inhibition of senescence, cell transformation
10. Alters cell proliferation, cell death or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

Abbreviations: AhR, aryl hydrocarbon receptor; ER, estrogen receptor; PPAR, peroxisome proliferator–activated receptor.

Any of the 10 characteristics in this table could interact with any other (*e.g.*, oxidative stress, DNA damage, and chronic inflammation), which when combined provides stronger evidence for a cancer mechanism than would oxidative stress alone.

ABBREVIATIONS AND GLOSSARY OF TERMS

3D	Three-dimensional
3R	Refinement, Reduction, Replacement
3T3 NRU PT	3T3 Neutral Red Uptake Phototoxicity Test
A[32]	Estimated daily exposure amount per kg body weight used in calculation of SED (%)
A	Androgen
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)
AHA	Amphibian Metamorphois Assay
AIC	Akaike Information Criterion
A.I.S.E.	The International Association for Soaps, Detergents and Maintenance Products
Alternative methods	All those procedure 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
AR	Androgen Receptor
Art.	Article
AhR	Aryl hydrocarbon receptor
ATMs	Alternative test methods
ATP	Adenosine Triphosphate
ATP	Adaptation to Technical and scientific Progress
AUC	Area Under the Curve
BCOP	Bovine Corneal Opacity and Permeability
BCRP	Breast Cancer Resistance Protein
BMD	The Benchmark Dose 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	The BMD lower limit refers to the corresponding lower limits of a one-sided 95% confidence interval on the BMD.
BMDU	BMD upper limit
BMR	BenchMark Response
BrdU	5-bromo-2-deoxy-uridine
BSE	Bovine Spongiform Encephalopathy
BW	Body Weight
CAS n°	Chemical Abstracts Service registry number
Cat.	Category
CEL	Consumer Exposure Level
CEN	European Committee for Standardization
CERAPP	Collaborative Estrogen Receptor Activity Prediction Project

[32] Used in the calculation of the Systemic Exposure Dose

CFU	Colony Forming Unit
CHMP	Committee for Medicinal Products
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
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
ER	Estrogen Receptor
ERBA	Endocrine Receptor Binding Assay
ESAC	ECVAM Scientific Advisory Committee
ESDP	Endocrine Disruptor Screening Program
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
GR	Glucocorticoid receptor
GSTs	Glutathione S-transferases
GUM	Gesellschaft für Umweltmutationsforschung
Hair product	A cosmetic product which is intended to be applied on the hair of head or face, except eyelashes (2009/1223/EC)
HBM	Human Biomonitoring
HCA	High Content Analysis
HESS	Hazard Evaluation Support System
HET-CAM	Hen's Egg Test-Chorio Allantoic Membrane
HET-MN	Hen’s Egg Test for Micronucleus
HPG	Hypothalamus-pituitary-gonad

HPLC	High-Performance Liquid Chromatography
HPLC-PDA	High-Performance Liquid Chromatography/Photo-Diode Array detection
HPRT	Hypoxanthine-guanine PhosphoRibosyl Transferase
HPT	Hypothalamus-Pituitary-Thyroid
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
ICH	International Conference on Harmonisation
***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	Infra Red
IRE	Isolated Rabbit Eye
ISO	International Organization for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
JRC	Joint Research Centre
KE	Key event
kNN	k-nearest neighbour algorithm
LAGDA	Larval Amphibian Growth and Development Assay
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)
LOD	Level Of Detection
LOQ	Level Of Quantification
MDCK	Madin-Darby canine kidney cells
MDR	Multi Resistance Protein
MEC	Molecular Extinction Coefficient
MIE	Molecular Initiating Event
MLA	Mouse Lymphoma Assay
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
NAMs	New Approach Methodology
Nanomaterial	An insoluble or bio-persistent an 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
NESIL	No Expected Sensitising Induction Level
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
OCHEM	Online Chemical Modeling Environment
OD	Optical Density

OECD	Organisation for Economic Co-operation and Development
OPPTS	Test Guidelines for Pesticides and Toxic Substances
P_{50}, P_{90}	50^{th}, 90^{th} percentile
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
Personal care products	Consumer products used: for beautification (make up products) and in personal hygiene (shower gel, skin cream, shampoo, feminine hygiene products, diapers, toilet paper etc.)
PIF	Product Information File
PMS	Post-Marketing Surveillance
POD	Point of Departure
P_{ow}	n-octanol/water partition coefficient
PPD	p-Phenylenediamine
PPAR	Peroxisome proliferator-activated receptor
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)
PXR	Pregnane X Receptor
QMRF	QSAR Model Reporting Format
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
RP	Responsible person
RSMN	3D-human reconstructed skin micronucleus assay
SAF	Sensitisation Assessment Factors
SAR	Structure-activity relationship
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

SED	The Systemic Exposure Dose of a cosmetic ingredient is the amount expected to enter the bloodstream (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).
SD	Standard Deviation of the mean
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
S	Steroidogenic
S_9	Fraction (supernatant) containing cytosol and microsomes of cells after centrifugation at 9000g
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 is 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)
SPF	Sun Protection Factor
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
T	Thyroid
TEST	Toxicity Estimation Software Tool
TG	Test Guideline
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 th body and include absorption, distribution, biotransformati and/or excretion (ADME) (ECB, 2003)
TSE	Transmissible Spongiform Encephalopathy
TTC	Threshold of Toxicological Concern
UGTs	Uridine diphosphate Glucuronosyltransferases

Undesirable effect	An adverse reaction for human health attributable to the normal or reasonably foreseeable use of a cosmetic Product (2009/1223/EC)
UN GHS	United Nations Globally Harmonised System of Classification and Labelling of Chemicals
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
Xprt	Xantine-guanine PhosphoRibosyl Transferase gene
yH_2AX	Phosporylated for of H_2AX histone