SCCS/1647/22
Corrigendum 2

Scientific Committee on Consumer Safety

SCCS

THE SCCS NOTES OF GUIDANCE FOR THE TESTING OF COSMETIC INGREDIENTS AND THEIR SAFETY EVALUATION 12TH REVISION

The SCCS adopted this guidance document
by written procedure on 15 May 2023

The corrigendum 1 was adopted during plenary meeting on 26 October 2023, and the corrigendum 2 by written procedure on 21 December 2023

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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 11 March 2004; no *in vivo* testing for local toxicity after 11 March 2009 and no *in vivo* testing for repeated dose toxicity (including sensitisation) toxicokinetics and developmental toxicity from 11 March 2013 onwards for the purpose of cosmetics. For this reason, the SCCS has closely followed the progress made toward 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 2021 (SCCS/1628/21). 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/new approach methodology (NAM).
As was also the case in previous revisions, individual Opinions are not provided in detail but, where relevant, 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 with focus on the so-called Annex substances of Regulation (EC) N° 1223/2009.

Input of scientists from the Scientific Committee on Health and Environmental and Emerging Risks (SCHEER) and Cosmetics Europe (CoE) is gratefully acknowledged.

The Chairperson

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Register of Commission expert groups and other similar entities (europa.eu)

The SCCS Notes of Guidance document is not open for commenting as it remains a living document, which is regularly updated. Any observation may be sent to SCCS mailbox (SANTE-SCCS@ec.europa.eu) for further consideration by the SCCS.

- ➔ The corrigendum 1 on Table 3A page 27 (footnote added under deo non-spray) was adopted on 26 October 2023 and **the corrigendum 2 on Tables 3A and 3B was adopted on 21 December 2023.**

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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.
They 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 independent experts.

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

SCCS

The Committee shall provide Opinions on questions concerning 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.

SCCS - Opinions (europa.eu)

Applicants are invited to visit the SCCS website:
SCCS - Opinions (europa.eu) where Applicants can find all published Opinions.
They will also find a checklist
for submitting a safety dossier of a cosmetic ingredient:
Checklists for Applicants submitting dossiers on Cosmetic Ingredients to be evaluated by the SCCS (europa.eu)

Applicants are invited to visit the following website for further legislative information:
https://ec.europa.eu/growth/sectors/cosmetics/legislation_en

MAIN CHANGES IN 12TH REVISION OF THE SCCS NOTES OF GUIDANCE (NOG)

The NoG have been revised and updated with emphasis on the following:

- Importance of systematic literature review
- Updating of animal-free alternative methods: NAM (New Approach Methodology), changes introduced for acute inhalation, skin irritation testing, eye irritation testing with DAL (Defined Approach for eye irritation, Liquid), DASS (Defined Approaches for Skin Sensitisation), new *in vitro* methods for genotoxicity testing (3D skin Comet; *in vitro* micronucleus)
- Importance of AOP (Adverse Outcome Pathway), DAs (Defined Approaches), IATA (Integrated Approaches to Testing and Assessment), NGRA (Next Generation Risk Assessment) with definition of BER (Bioactivity/Exposure Ratio), TTC (Threshold of Toxicological Concern), iTTC (internal TTC)
- Updating of *in silico* prediction possibilities
- Exposure data reviewed (models, parameters specific for inhalation, aggregate exposure)
- Exposure of children to different cosmetic product categories according to age
- Sun protection by sunscreen products: rationale behind exposure data
- Human biomonitoring (HBM) and differences with SCCS approach for risk assessment
- CMRs reporting requirements
- Endocrine active substances, introduction of non-monotonic dose response, reporting requirements
- Templates for PBTK (Physiologically Based ToxicoKinetics) model description and parameter verification and analysis

1. INTRODUCTION

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

For ingredients which might pose a risk 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, exposure via relevant exposure routes, *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 has been applicable since 11 September 2004; the testing ban on ingredients or combination of ingredients has been applicable since 11 March 2009. The marketing ban has been applicable 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 has been applicable 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.

The present update includes the latest validated methods of the 3Rs (Refinement, Reduction and Replacement) (Russell and Burch, 1959), with emphasis on Replacement and 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. New methodologies for the risk assessment of chemicals without using animal experimentation are being explored worldwide. Attention is given here to Next-Generation Risk Assessment (NGRA) as a possible framework for the safety evaluation of cosmetic ingredients and the NAMs that would fit into this structure (Rogiers *et al.,* 2020). Risk assessment of cosmetics and their ingredients is shifting towards a strategic combination of NAMs and new technology with historical animal data, when available, to come to a Weight of Evidence (WoE) decision-making approach.

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 European Chemicals Agency (ECHA) can request animal studies even if the substance being studied is foreseen only for cosmetic use (see **Appendix 1**, section 3). The applicant can submit these animal data to ECHA, but cannot use these in the cosmetic product safety report (CPSR) for the product information file (PIF) and cannot submit these to the SCCS for risk assessment of the ingredient under consideration. SCCS can ask ECHA for access to these studies and consider whether the results have an impact on the risk assessment of the substance and change their view.

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 BACKGROUND

The Commission Decision C(2015)5383 of 7.8.2015[1] established the new Scientific Committees in the field of public health, consumer safety and the environment. Members were appointed[2] for a five-year term (2016-2021) and a reserve list[3] was created. The term was extended until end of 2026 due to Covid-19. The Principles and Working Procedures of the Scientific Committees are stated in their establishing Decision and in the Rules of Procedure adopted by their members (April, 2016)[4].
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 of minimum four weeks (later agreed on eight weeks) 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.

[1] https://ec.europa.eu/health/sites/health/files/scientific_committees/docs/call_2015_5383_decision_with_annexes_en.pdf
[2] https://ec.europa.eu/health/sites/health/files/scientific_committees/docs/appointment_letter_2016_en.pdf
[3] https://ec.europa.eu/health/sites/health/files/scientific_committees/docs/appointment_reserve_list_2016_en.pdf
[4] https://ec.europa.eu/health/sites/health/files/scientific_committees/docs/rules_procedure_2016_en.pdf

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 11th Revision SCCS/1628/21 is now replaced by the 12th Revision.

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. They 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/NAMs 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.
As regards data generated after the deadlines of the testing and marketing bans, see Section 3 of **Appendix 1**.

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 companies/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 to be used exclusively 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/NAMs.
- When 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 to be used also in products other than cosmetics (*e.g.* food, medicines, biocides, etc.) can be used for supporting the safety assessment of an ingredient intended to be used in a cosmetic product.

  Further information is provided in Section 3 of **Appendix 1.**

When information from the scientific literature regarding toxicological domains is used in the NoG, **a systematic search and review following pertinent scientific standards** (*e.g.* PRISMA) (Shamseer *et al.,* 2015) **should be performed, documented and submitted as part of the dossier** (PDF for all references quoted, search engine used for

literature search, key words, reason for inclusion or exclusion). Specific considerations for assessment of quality and risk of bias depend on the toxicological endpoint and type of study, respectively. These considerations are addressed in the specific sections of these Notes. In general, the criteria laid down in the NoG and the OECD testing guidelines should be used as the benchmark. To assess the quality of a toxicological study, the Klimisch score (Klimisch et al., 1997) could be used, for example. A software-based tool named 'ToxRTool' has been developed by the European Union Reference Laboratory for the Validation of Alternative Methods (EURL ECVAM) (Schneider *et al.,* 2019) to determine the Klimisch score in a systematic way (also suitable for *in vitro* studies). The tool can be downloaded via the EU Science Hub of the European Commission website.

IMPORTANT REMARKS:

- **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.**

- **The preliminary Opinions published by the SCCS are meant to invite comments and suggestions for finalisation of the specific Opinions. Therefore, the commenting period must not be considered an opportunity for an Applicant to submit a new dossier.**

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, 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**).

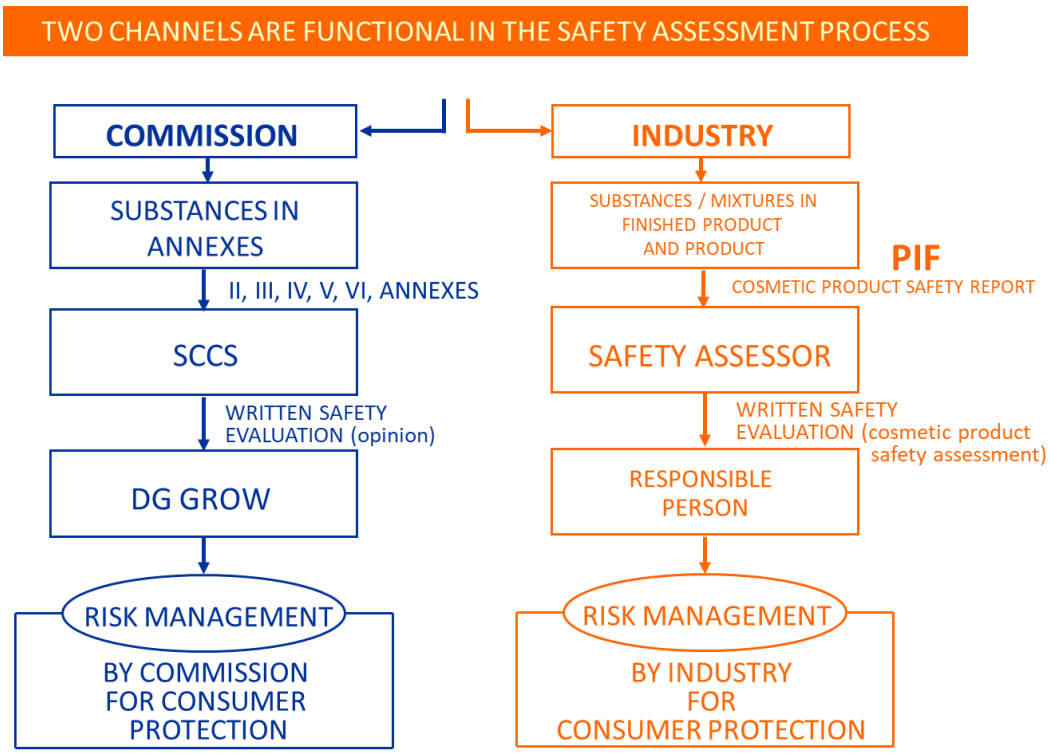


Figure 1: Human health safety evaluation of cosmetic ingredients in the EU.

PIF: Product Information File

- **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 that is universally applied for chemical substances, with the stipulation that only validated replacement methods (or those 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* studies, *in vitro* and *ex vivo* tests, *in chemico* methodology, *in silico* methods and read-across, clinical studies, case reports, epidemiological studies and data from 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 a 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 women, 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 Authority (EFSA) and the World Health Organization (WHO) also recommend using the BMD approach for deriving the PoD as a starting point for human health risk assessment.

In the BMD modelling, the dose-response relationship follows an increasing or **monotonic trend**. This means that the higher the exposure to a hazardous compound, the higher the probability of an effect occurring (for probabilistic effects) or the severity of the effect (for deterministic effects).
The BMD approach has a number of advantages over using NOAEL:

- 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 BM, Lower limit (BMDL) (for details of the NOAEL and BMD approaches, see Sections 3-4.8, 3-5.1).

Conversely, for some compounds, a so-called "**non-monotonic"relationship** has been observed that may bend at a particular point on the curve (**Figure 2**).

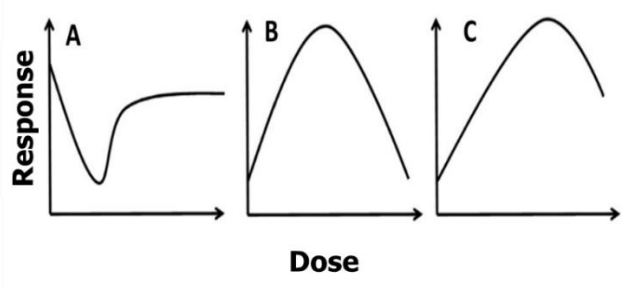


Figure 2: Examples of non-monotonic dose-response relationships. A: example of a U-shaped curve where the inflection point appears in the low dose area. B: example of an inverted U-shaped or bell-shaped curve. C: example of a non-monotonic curve where the inflection point appears in the high-dose area

Receptor saturation phenomena or a sequence of agonist/antagonist effects (Conolly & Lutz, 2004; Lagarde *et al*., 2015) could, for example, lead to this type of relationship. This non-monotonicity has been discussed for a number of substances with **endocrine disrupting potential**. The study of these substances over wide dose ranges, including very low doses, has indeed shown inflection points in the dose-response curve. However, this is not always a non-monotonic dose-response relationship but sometimes an experimental artifact or a visual observation not supported by appropriate statistical analysis (Varret *et al.,* 2018).

Determining a no-effect dose becomes complicated. One possibility is to divide the non-monotonic curve into many portions of monotonic curves and, for those in the range of doses equivalent to the exposure doses, to determine an effect threshold. However, care must be taken when deriving reference values, which often leads to the application of safety factors, to ensure that the reference value does not lie in a portion of the curve where effects at very low doses would be possible. Another approach would be to consider these compounds as "non-threshold" compounds and thus to apply a probabilistic approach, as for genotoxic carcinogens. In this case, it is also necessary to isolate the portion of the curve corresponding to the exposure levels and to calculate the unit excess risk in this area.

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 available[5]. In the case of an oral toxicity study, the following equation **(1)** is used:

$$MoS = \frac{PoD_{sys}}{SED} \quad (1)$$

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 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 or the RP, the latter when a finished cosmetic product and its ingredients are involved (**Figure 1**).

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 and/or Word and need to be readable and searchable.

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

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.

An example of the framework of a typical dossier is given in **Appendix 3.**

The contact point for dossier submissions and regulatory/risk management questions is: GROW-F2@ec.europa.eu
The SCCS address for scientific requests related to published opinions is: SANTE-SCCS@ec.europa.eu

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 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 3-6.8). Original data on all these points must be included in each toxicological dossier and information and documentation for all analytical data should be provided.
The appropriate certificate of analysis must also be presented 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, 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 3-6.8 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 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 High Performance Liquid Chromatography (HPLC) peak area can only be accepted when:

1) a reference material of known purity is used,
2) the HPLC recovery of the test material is clearly documented,
3) the ultraviolet (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 UV, InfraRed (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 the 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.

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, (OECD GD123 and OECD GD 117)

- 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-absorbing substance, the UV-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 3-6.8).

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. In **Table 2**, different solubility terms have been defined.

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 another 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.

Table 2: Definition of solubility terms (adapted from US Pharmacopeia, National Formulary (USP38/ USP38–NF33* and General Notices) and European Pharmacopoeia (Ph. Eur. 11th Ed.)

Term*	**Parts of Solvent Required for 1 Part of Solute***	**Solubility defined in g/l** (deduced by SCCS)
Very soluble	Less than 1 part	>1000
Freely soluble	1 to 10 parts	100-1000
Soluble	10 to 30 parts	33.3-100
Sparingly soluble	30 to 100 parts	10-33.3
Slightly soluble	100 to 1000 parts	1-10
Very slightly soluble	1000 to 10000 parts	0.1-1
Practically insoluble, or insoluble	>10000, or equal to10 000 parts	< 0.1 or = 0.1

*Under USP38/ USP38–NF33: practically insoluble is used in USA; in EU: insoluble

3-2.8 Partition coefficient (Log Pow)

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 products 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.* rolled-on, 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. These are mostly 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).

3-3.3.1 Local external dose

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).

For the evaluation of a local effect in the lung, 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 (see cyclopentasiloxane D5 Opinion SCCS/1549/15).

For skin sensitisation, see Section 3-4.7.1.

For skin irritation, see Section 3-4.5.2. Skin irritation is to a large extent dose-dependent; in relevant circumstances the SCCS will evaluate this on a case-by-case basis.

For eye irritation, *in vivo* tests are not anymore allowed for cosmetic ingredients. Although most *in vitro* tests only distinguish between 'serious eye damage' and 'no classification needed' (Kaluzhny & Klausner, 2021), new developments make it possible to distinguish between the 3 categories (see 3-4.6).

If a local effect on the eye exposure of the eyelids can theoretically be assumed, then the SCCS will evaluate this on a case-by-case basis. An example is present in SCCS/1635/21 (prostaglandins and analogues 2022).

Local effects in the oral cavity, including irritation/corrosion of the gingiva, tooth sensitivity and tooth enamel erosion, may occur when extreme low or high pH values are present.

3-3.3.2 Systemic dose

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 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 **in a first tier** 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 second 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. 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:

1) 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.
2) The target protection goal will be the 95th percentile of the European population. Therefore, for a probabilistic assessment of the relevant SED for deriving the MoS, the 95th percentile of the probabilistically assessed population exposure will be used.
3) 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.
4) The exposure assessment normally should assume 100% occurrence probability (i.e. in each product category where the cosmetic ingredient is allowed, it is present). This conservative assumption is needed because (i) there may be a dependency in product use that cannot be covered by the probabilistic model (*e.g.* a person may have a preference for a specific brand that always uses the same UV-filter) and (ii) because occurrence levels may change. If another occurrence probability is used, it needs to be carefully substantiated and specific data are required.
5) 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.
6) Commercially available models (*e.g.* the Crème model, often used by the cosmetic industry) (Mc Namara *et al.*, 2007) are not publicly accessible, which makes the evaluation of the exposure assessment more difficult. Therefore, in order to ensure the transparency needed for regulatory purposes, the used model parameters have to be listed (ideally in the form of a structured input file) together with the referenced/substantiated and submission of detailed results, ideally together with an

output file. The required parameters are listed in a checklist given in **Appendix 14** (not exhaustive). In addition, information on the distributions used is necessary.

7) A publicly available model, *e.g.* PACEM, was recently developed into a web tool, www.pacemweb.nl. It only includes product usage data on personal care and household cleaning products, obtained in surveys from EU countries. Also, exposure fractions need to be provided as user input which can be derived via the ConsExpo tool, www.consexpoweb.nl (Delmaar *et al*., 2022).

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 **Tables 3A and 3B** 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:

$$E_{dermal\,x} = C_x \times q_x \times f_{ret\,x} \qquad \textbf{(2)}$$

$E_{dermal\,x}$ (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 for dermal exposure per product category, $E_{product} = q_x \times f_{ret\,x}$, which is listed in **Tables 3A and 3B** 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 $E_{dermal\,x}$ shown in equation **(2).**

This external exposure can be used to calculate the SED by multiplying with the chemical- and route-specific uptake rate and normalisation by the body weight (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 4**) 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. Furthermore, 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 Skin Surface Area of application (SSA).

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 small.

3-3.4.1.3 INHALATION EXPOSURE MODELS

Cosmetic substances can be inhaled either after evaporation from the location of use, or when used in spray applications, or in the form of a powder. Evaporation is only relevant for volatile substances, whereas after spray or powder application,non-volatile substances are also transferred into the air as aerosolised droplets and/or aerosolised particles. It should be noted that after muco-ciliar clearance of the inhaled fraction, further intake of insoluble particles or their components *via* the oral route may occur.

External exposure to vapour can be calculated directly based on measurement of the concentration of the substance in the air. For inhalation exposure to substances in sprays and powder, the assessment needs to take into account the particle size distribution of the aerosolised particles and droplets after application and the respective deposition rates in different parts of the lungs, (*i.e*. depends on how deep can the particles penetrate the lung). In the safety evaluation of sprays and powders, the robustness of the exposure data therefore plays a major role (Steiling *et al*., 2018).

The deposition efficiency in the respiratory tract is not only size-dependent but also depends on the form (spheric or other), density, electrostatic properties 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). Part of these are influenced by the presence/absence of surface coatings on the particles. However, particle and droplet size is generally regarded as the most important influencing factor for deposition and penetration of the various lung areas. The size of the particles/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. It is also closely related to the geometry of the spray nozzle and the can size.

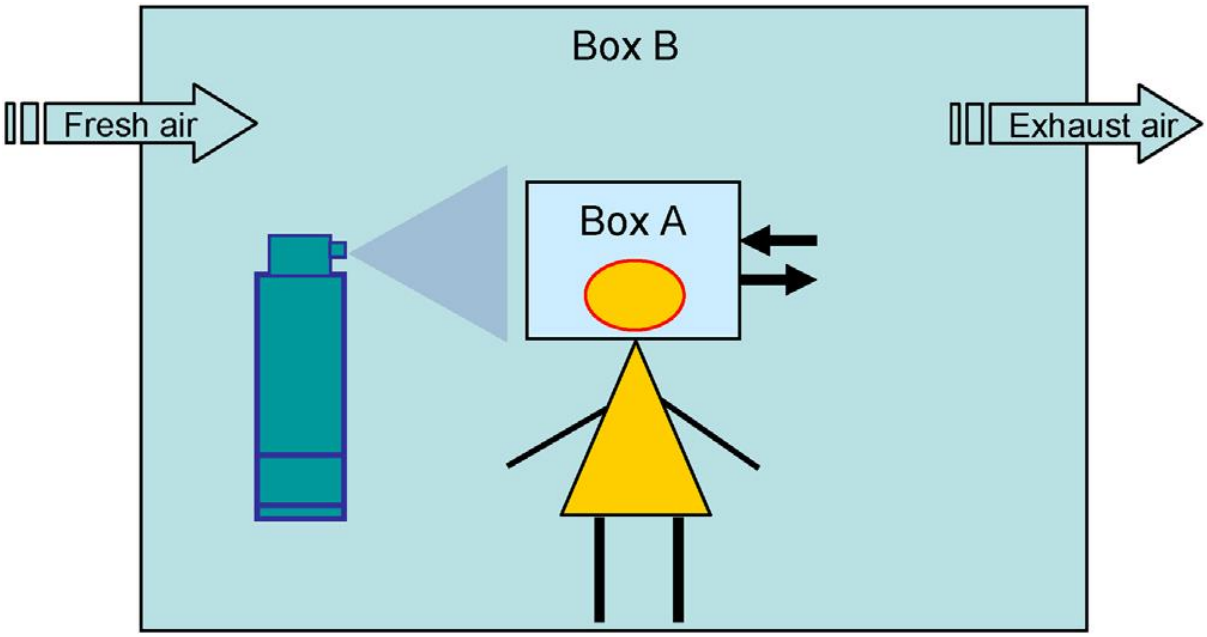
The size fraction comprising droplets/particles with a Mass Median Aerodynamic Diameter (MMAD) of ≤100 µm is generally regarded as inhalable, while the thoracic and respirable fraction with a MMAD of ≤10 µm or ≤ 5µm, respectively, are generally regarded as small enough to reach the deep part of the human trachea and the lungs, where droplets/particles can enter the alveoli and may be taken up and become systemically available (Snipes, 1989; Valentine and Kennedy, 2008). In animals (*i.e*. rodents that inhale and exhale through their
nostrils, these values are lower due to smaller dimensions of the respiratory tract and 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 workplaces (CEN, 1993). Estimates for adults and children during typical activities with both nasal and oral inhalation have been determined by Brown *et al.* (2013).

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

droplets. However, also for pump sprays, the size of the droplets produced depends on the pump nozzle. Studies by *e.g.* Quadros and Marr (2011) have shown that pump nozzles can even produce particles/droplets in the nano size range (i.e. <100 nm). Another important consideration in relation to the airborne aerosols is that they can change their number and size distribution with time (*e.g.* by aggregation of particles and evaporation of solvent) before they reach the airways. Thus, they can 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 the potential drying process and their resulting size distribution just before inhalation and deposition. Furthermore, when measuring exposure, it is important to record it during the relevant exposure period after spraying, under relevant conditions (Carthew *et al.*, 2002; Rothe *et al*., 2011). This is especially important for spray/sprayable cosmetic products containing nanomaterials, but also relevant for larger particles/droplets. For more detailed considerations on nanoparticles and droplets, see the Guidance on the Safety Assessment of Nanomaterials in Cosmetics (SCCS/1611/19).

The level of exposure can be estimated by using mathematical models or be directly measured under standard exposure conditions. When using mathematical models, a tiered approach should be followed. Default equations can be used as a conservative, worst-case approach, and as a first estimate (ECHA, 2012b). For a more realistic assessment, 1- or 2-Box models, as well as higher tier models, can be considered. In a classical 1-Box model it is assumed that the entire spray amount is instantaneously released into the air and distributed in a box of specific size, which e.g. simulates the breathing zone (Box A in Figure 2). The resulting air concentration is then multiplied by the breathing rate and the time spent in the box to calculate the exposure. A 2-Box model takes into account the dilution of the substance over time. As in the 1-Box model, the assumption is that the spray is instantly released and distributed in a box around the head, Box A. There, the aerosol is present for exposure over a defined time, after which the full amount of aerosol in the first box is transferred to a larger second box, Box B (see **Figure 3**), where it is available for inhalation for a second defined time period. For a conservative approach, the air exchange between Box B and the surrounding environment (fresh air getting in, exhaust air getting out) should be assumed as zero. An example of a 2-Box model assessment is given in Rothe *et al*. (2011).

Figure 3: Deterministic 2-Box model (according to Steiling *et al.,* 2014); copyright from Elsevier, first published in Toxicology Letters 227, 2014.



For higher tier assessment, one of the tools that can be considered for calculating exposure estimates is the ConsExpo model (www.consexpo.nl). In ConsExpo, 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" (rate at which mass is released by spraying), airborne fraction, aerosol size distribution, and weight fraction of the ingredient. The 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 (mass-based). For conventional (non-nano) substances, it is assumed that these are homogeneously distributed in the box through the generated aerosols. Since nanoparticles had not been measured in the calibration data set underlying the model, **ConsExpo Spray cannot be used directly for nanoparticles.** For nanoparticles in spray products, the Consexpo Nano tool can be used (Bremmer *et al.,* 2006b).

Inhalation is not the intended route of exposure for cosmetic exposure. Therefore, the flow chart (see **Figure 4**) can be followed to determine whether assessment of inhalation exposure is necessary for a given cosmetic formulation.

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 percentil
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.

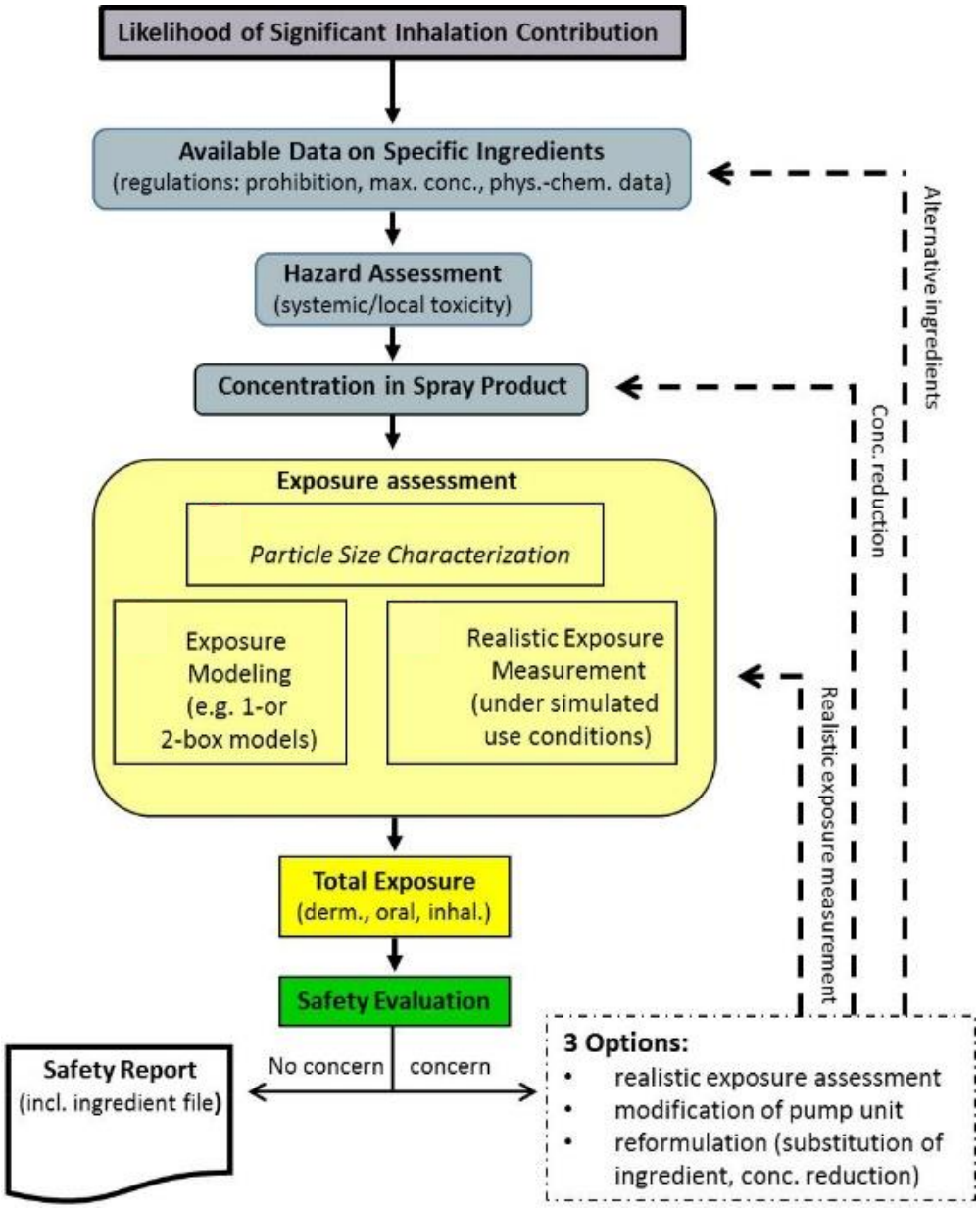


Figure 4: Basic principles for the tiered safety assessment of inhalable cosmetic products and their ingredients. Modified from Steiling *et al.,* (2014); grey = related to ingredients; yellow = related to product exposure.

3-3.4.2.1 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. These Member States were Spain, Great Britain, France, Germany and Denmark, where the population of Spain represented the populations of southern European countries, such as Spain, Italy, Portugal and Greece; Great Britain represented those of the United Kingdom and Ireland. The population of France represented only the one of France, whereas the population of Germany represented mid-European countries such as Germany, Belgium, Luxembourg, the Netherlands and Austria. The population of Denmark represented the northern European countries i.e. Denmark, Finland and Sweden. 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 3A** 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 ($E_{product}$). For deriving the relative amounts and exposures reported in **Table 3A**, bodyweight distributions from the European countries included in the study were used in a Monte Carlo approach explained in Hall *et al*., 2007 and McNamara *et al*., 2007.
From the $E_{product}$ derived below, the dermal exposure E_{dermal} to a substance can be calculated according to equation **(3)**:

$$\mathbf{E_{dermal} = E_{product} \; X \; C_x} \qquad \textbf{(3)}$$

where C_x: substance concentration in a product category.

Table 3A: Daily exposure levels for different cosmetic product categories in Europe, calculated by multiplying daily amounts (Hall *et al*., 2007, 2011) and f_{ret}.

Product type	Estimated daily amount applied q_x (g/d)	Relative daily amount applied[1] q_x /bw (mg/kg bw/d)	Retention factor[2] f_{ret}	Calculated daily exposure $E_{product}$ (g/d)	Calculated relative daily exposure[1] $E_{product}$ /bw (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	57.4	0.10	**0.40**	**5.74**
Skin care					
Body lotion	7.82	123.2	1.00	**7.82**	**123.2**
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[3]	6.54	93.7	1.00	**6.54**	**93.7**
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.
[3] For **deodorant spray**, the exposure values were obtained by measuring the product leaving the can, thus this value includes dermal deposition and the inhaled fraction, which was not determined separately. Steiling *et al.*, 2012 have derived worst case exposure fractions for dermal exposure that can be applied to derive dermal exposure estimates (23.5 % and 11.4% for ethanol-based and non-ethanol-based sprays, are available for dermal exposure, respectively).

The large-scale study cited above only included the most frequently used 12 cosmetic products. Deterministic amounts and exposure data for further cosmetic products had been provided earlier for normal and extensive use (Colipa 16.01.97 BB-97/007, SCCNFP /0321/00). **Table 3B** lists conservative use levels for some cosmetic products based on female usage (higher than for males) and extensive use reported by SCCNFP.

Table 3B: Estimated daily exposure levels in Europe for additional cosmetic product categories, which are not covered by Hall *et al.,* 2007, 2011 (SCCNFP/0321/00; Colipa 16.01.97 BB-97/007).

Product type	Estimated daily amount applied q_x (g/d)	Relative daily amount applied[4] q_x /bw (mg/kg bw/d)	Retention factor[1] f_{ret}	Calculated daily exposure $E_{product}$ (g/d)	Calculated relative daily exposure $E_{product}$ /bw (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)	-	0.1	**Not calculated[3]**	**-**
Oxidative/permanent hair dyes[2]	100 ml (per application)	-	0.1	**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**

[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). Being a fraction between 0 and 1, f_{ret} has no units.
[2] Product categories not covered by Hall *et al*., 2007, 2011.
[3] Daily exposure value not calculated due to the low frequency of application.
[4] The specific body weight of the persons involved is used and not the default value of 60 kg.

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, the surface area of body parts, for example, can be helpful. Therefore, in **Table 4,** 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 default human body weight of 60 kg.

Table 4: 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 for application **SSA** (cm^2)	Body areas	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 If 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 (SSAs) 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 the 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 are default values 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 - both propellant driven aerosol and pump sprays - the relevant concentration to calculate exposure is not the concentration in the formulation, but the concentration in the spray mist, which can be inhaled (3-3.4.1.3). The droplet size distribution should also be considered. Finally, according to the explanations in 3-3.4.1.3 (inhalation models), another important parameter is the deposition of the amount of substance via particles/droplets in the lung.

In **Appendix 11**, different models to estimate the total and regional lung deposition of aerosol and/or particles are provided.
Also, possible parameterisation for a 2-Box inhalation model is given as an example and needs to be adapted to the specific exposure scenario.
Taking into account the small timeframe of the calculation and large variation in room ventilation, for a conservative estimate, it should be assumed that no ventilation occurs.

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 when 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 (*e.g*. which could be 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, the external exposure needs to be aggregated over product categories. If additionally, aggregation over routes is necessary because different routes (*e.g.* dermal and inhalation route) contribute, aggregation over routes needs to be calculated on the level of internal exposure. In this case, the aggregation over routes needs to be conservative. This means that if one exposure route is more effective than the other (*e.g.* the inhalation route results in more uptake than the dermal route) the highest possible fraction should be attributed to the most effective route of exposure (the one with the highest uptake). The rest (highest fraction subtracted from the rest) should be attributed to the other route(s). If it is not straightforward to decide which route contributes most, several scenarios can be calculated.

As a first tier aggregate dermal exposure assessment, the SCCS recommends calculating the LEDs and SEDs based on the product category-specific exposures $E_{product}$ given in **Table 5**. For preservatives and other substances that are regulated with the same maximal concentrations in all product categories, the LEDs or SEDs can be directly 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 5** were taken from the $E_{product}$ presented in **Table 3A.** For some product categories probabilistic data were not available and for these categories earlier information provided by Cosmetics Europe was used (**Table 3B**). 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.

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 3A, 3B, 4 and 5** since the inhalation and oral risk is assessed on a case-by-case basis.

For CMR 1A and 1B substances, according to Art. 15d of the Cosmetic Regulation, safety evaluation needs to take aggregate exposure from all sources (including non-cosmetics) into consideration (see **Appendix 5** and **3-6.5**). Safety evaluation should also include children according to age.
For compounds evaluated by the SCCS as having potential endocrine activity, safety evaluation of children should be included for the relevant cosmetic categories to which children of different ages usually are exposed to (a proposal of cosmetic product categories is shown in **Appendix 7, Table A.7.2**).

When aggregate exposure is calculated for the different product categories and the MoS is <100, then the industry should decide whether all concentrations are lowered in concentration, or one (or more) particular product category(ies) is (are) taken out.

Table 5: Product exposures for the deterministic calculation of aggregate exposure for preservatives through cosmetic use. Note that these values can also be used for other ingredients when aggregate exposure calculations are needed for one or more classes of cosmetic products.

Type of cosmetic product exposure	***Product category***	***Daily Exposure*** $E_{product}$ *(g/d)*	***Relative daily exposure*** $E_{product}$ **/bw** [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
Aggregate exposure		**17.4**	**269**

1. The specific bw 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)

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 values that translate the amount of substance entering the body into the amount that is available in the bloodstream 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 these processes need to be known to understand the fate of substances once they come in contact with 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 TG 417 (toxicokinetics), TG 427 (*in vivo* method), TG 428 (*in vitro* method), 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.

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).

A review of the current status of toxicokinetics in the safety evaluation of cosmetics and their ingredients can be found in the scientific literature and especially in several JRC reports (Adler *et al.,* 2011, JRC Scientific and Policy Report 2013a, 2014a, b, 2015, 2016, 2017 (more specific to toxicokinetics), 2018-2022). 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 and Zuhang*,* 2005; JRC Scientific and Policy Report 2013a, 2014a, 2014b, 2015-2022).

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), salicylic acid (SCCS/1601/18) and aluminium (SCCS/1613/19). 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 (US Environmental Protection Agency), 1996a; OECD GD 28; WHO, (World Health Organisation) 2006; OECD GD 156, EFSA 2017; SANTE 2018). Sometimes, different terminology is used.

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

Skin absorption studies can be performed *in vitro* (OECD TG 428) or before the testing deadlines *in vivo* (OECD TG 427). Detailed guidance on their performance is available (OECD GD 28, OECD GD 156), although no OECD test guideline is available to describe how to conduct *in vivo* human dermal absorption studies. In addition, the SCCNFP

(Scientific Committee on Cosmetics and Non-Food Products) adopted a first set of "Basic Criteria" for the *in vitro* assessment of dermal absorption of cosmetic ingredients (SCCNFP/0167/99), which was later updated in SCCS/1358/10. A combination of OECD TG 428 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:

- The design of the diffusion cell (technicalities and choice between static and flow-through system).
- The choice of the receptor fluid (physiological pH, solubility and stability of chemical in the receptor fluid should be demonstrated, no interference with skin/membrane integrity, analytical method, etc.).
- 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).
- 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).
- Skin temperature has to be ascertained at normal human skin temperature.
- 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.
- 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.
- Regular sampling is required during the entire exposure period, taking into account delayed penetration into skin layers.
- 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

1) product excess on the skin surface (dislodgeable dose),
2) *stratum corneum* (*e.g.* adhesive tape strips),
3) living epidermis (without *stratum corneum*),
4) dermis,
5) receptor fluid.

- 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%.
- An appropriate number of controls (for *in vitro* studies: diffusion cells) should be used to determine the background level. In cases where there is a high background level and a high variability of the background level, it may be necessary to determine it for every single donor in an appropriate number of repetitions.
- 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. 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.

- 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.
- 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 GD 28; OECD GD 156; Van de Sandt *et al.*, 2004).
- 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, a 50% default value is used.** This conservative value may also be used in cases where only inadequate dermal 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.* hair dyes 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 clearly 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** (Standard Deviation) 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 will not be accepted and will be excluded; a 50% default value will be used when no other data become available.

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.

For all *in vitro* methods in general, the Guidance Document on Good *In Vitro* Method Practices (GIVIMP) captured as OECD GD 286 should be followed. GIVIMP provides guidance to users and implementers of *in vitro* methods, giving a detailed update on good practices for state-of-the-art *in vitro* methods and describing key aspects that may impact the reliability and relevance of the *in vitro* data for quantitative human safety assessment purposes.

c. Substances with very low dermal absorption

A retrospective study of the Annex substances presented 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):

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

For dealing with data on very low dermal absorption, see Section 3-6.11.

d. In silico estimation of dermal absorption

In silico models and tools can provide another means for gathering supporting information on the skin permeability of chemical cosmetic ingredients. A number of QSAR models have been developed for this purpose (Cronin *et al.,* 2022). The prominent ones amongst these are the 'ten Berge' model (ten Berge, 2009) and the 'Potts and Guy' model (Potts and Guy, 1992) that can be used to estimate skin permeability coefficient (Kp), which is the rate of a chemical penetrating across the skin (cm/h).

The 'ten Berge' model is based on regression of the data from human skin *in vitro* from aqueous solutions, while the 'Potts and Guy' model is based on physiologically based kinetic/dynamic parameters from data on hairless mouse skin tests carried out according to OECD 428 test guidelines. Both models have been incorporated in some QSAR systems – such as SpheraCosmolife (Selvestrel *et al*., 2021) – now called Vermeer Cosmolife – which also provides an indication of the reliability of the estimates by showing whether or not the query substance is within the applicability domain of the model.

In this regard, a useful calculator (SkinPerm) has been developed by NIOSH/CDC to estimate skin permeation of chemicals (www.cdc.gov/niosh/topics/skin/skinpermcalc.html).

It estimates the skin permeation coefficient (Kp) from an aqueous vehicle using three different models – Frasch (2002), Potts and Guy (1992), and Modified Robinson (Willschut *et al.*, 1995) - that have been optimised based on the experimental data on Kp compiled in the associated Flynn database (1990). (It requires 3 inputs - molecular weight, logKow, and maximum concentration (*i.e.* aqueous solubility). The CAS number of a substance can also be used as an input if it is present in the Flynn database. The outputs of the calculator include Kp, LogKp, and Flux (Jmax). They can be used to estimate the % dermal absorption values for a given chemical.

3-3.5.1.2 ABSORPTION AFTER INGESTION

For products intended **for oral use**, like toothpastes and mouthwashes, some amount will inevitably 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. 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, (droplet/particle) aerosols or powders and enter the respiratory tract. The physical form of the ingredient plays a decisive role in the absorption process. In addition to size for particle-like substances, the surface characteristics including coating are important as well. 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 dissolution 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, soluble particles dissolve (or dissolve partially) in the lung lining fluid (mucus layer) of the epithelium whereas inert particles might form non-dissolved but colloidal suspensions. For further considerations of particle behaviour, refer to the Guidance on the Safety Assessment of Nanomaterials in Cosmetics (SCCS/1611/19).

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. The dose used as starting point for the risk assessment should be either the external exposure dose in the air surrounding the user or the calculated/modelled dose as deposited in the lung, using the external air exposure as starting point.

3-3.5.2 DIFFERENCES IN METABOLISM FOR DIFFERENT ROUTES

3-3.5.2.1 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; Kazem *et al.,* 2019).

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 regarding 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; Kazem *et al.,* 2019). 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.

In the future, these skin models may help 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, 2016).

3-3.5.2.2 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. As in skin, the expression of xenobiotic metabolising enzymes in the lungs 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 and serous cells. Cytochrome P450 (CYP) enzymes involved in xenobiotic metabolism have been identified in lung tissues from different species including humans (overview, Gundert *et al.,* 2014; Oesch *et al.,* 2019).

They can vary considerably between humans. Amongst conjugating enzymes, Glutathione S-Transferases (GSTs), Uridine diphosphate Glucuronosyl Transferases (UGTs) and arylamine-N-AcetylTransferases (NATs) have been identified, as well as, partially, their local distribution in the lung. Other enzymes present in lung are epoxide hydrolases and certain transporters such as Multidrug Resistance Proteins (MDR1 and MRP1) or Breast Cancer Resistance Protein (BCRP) (Gundert-Remy *et al.,* 2014).

3-3.5.2.3 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 3-5.1.3). 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 as possessing different carcinogenic potencies due to the different metabolisms of laboratory species compared and 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 SCCS/1443/11 and 6-Amino-m-cresol 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 & Eglen, 2017; Chen *et al.,* 2018).

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 Physiologically Based PharmacoKinetic (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 so from humans, is helpful or even indispensable in order to clarify if or to which extent relevant metabolites are formed (see OECD TG 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 be in compliance with the Memorandum on the use of human data (SCCS/1567/15).

3-3.5.3 PBPK MODELLING

PBPK models are quantitative descriptions of the Absorption, Distribution, Metabolism and Excretion (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 (WHO, 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, 2010).

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

A. High: ≥ 0.5
B. Medium: 0.2 ≤ medium< 0.5
C. Low: 0.1 ≤ low< 0.2

Uncertainty analysis is important and **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, 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 5** (WHO, 2010).

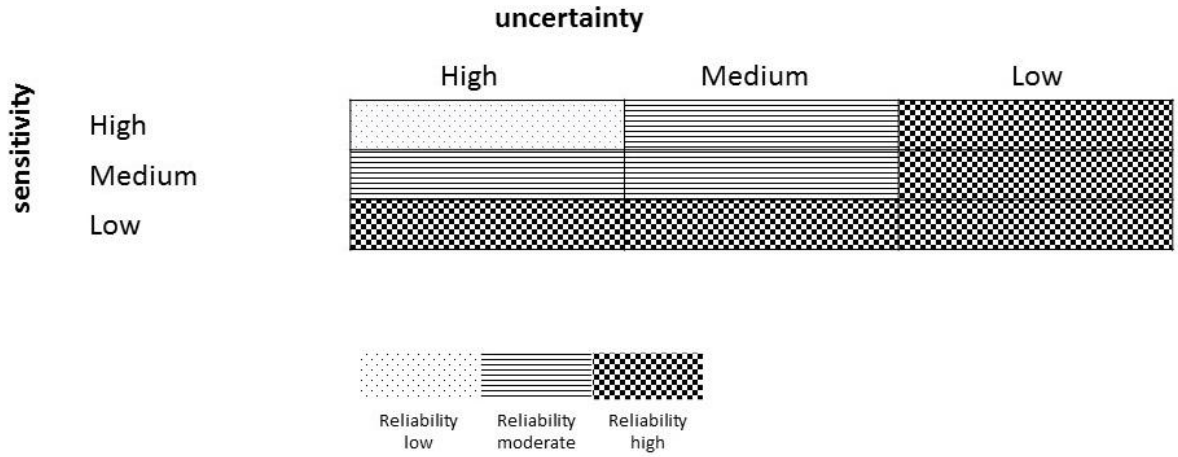


Figure 5: Illustration of the role of sensitivity and uncertainty analyses in determining the reliability of PBPK model predictions of dose metrics for safety evaluation (WHO, 2010).

Note that uncertainty and sensitivity analyses 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, 2010).

When estimated data from PBPK models are submitted to SCCS that 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 information is needed:

i. 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;

ii. Model parameterisation that involves obtaining quantitative estimates of measures of the mechanistic determinants (*e.g.* anatomical, physiological, physicochemical, biochemical parameters);

iii. Mathematical and computational implementation;

iv. Model simulation, *i.e.* simulation of the kinetics;

v. 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.

OECD has developed a guidance document on Physiologically Based Kinetic (PBK) models that provides insights into the way the data generated by such methods can be applied to construct PBK models and how these models can be validated (OECD GD 331). In **Appendix 13**, templates are presented to provide the information requested for PBTK model description in **Table 13.1** and the informationfor parameter verification and analysis in **Table 13.2**.

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

The SED 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).

Calculations of the SED

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 SSA needs to be known per product type (see **Table 4** 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 3A** and **Table 3B** 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 **(4),** 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).

$$SED = \frac{DAa \times 10^{-3} \times SSA \times f_{appl}}{60kg} \qquad \textbf{(4)}$$

Where:

SED (mg/kg bw/d)	Systemic Exposure Dose
DA_a (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 4** in Section 3-3.4.2 for SSA values per product type)
f_{appl} (day^{-1})	Frequency of application of the finished product
bw (kg bw)	human body weight (default value: 60 kg)

2) Dermal absorption reported as a percentage of the amount of substance applied **(5)**:

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.

$$SED = E_{product} \times \frac{C}{100} \times \frac{DA_p}{100} \qquad \textbf{(5)}$$

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 3A** and **3B**, 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 3A** and **3B**, the SED for the respective product category will have to be adapted accordingly

3-3.5.4.1 CALCULATION OF THE INHALATION SED_{INH}

Inhalation can occur after volatilisation of a dermally applied substance or after spraying a pump spray or propellant driven aerosol spray.
For calculating inhalation exposure to a substance after volatilisation **(6)**, the daily product exposure can be multiplied by the substance concentration and a suitable, conservative evaporating fraction (the worst-case parameter value for SED_{inh}) would be 1.

$$SED_{inh} = E_{product} \times \frac{C}{100} \times f_{evap} \qquad \textbf{(6)}$$

With

$E_{product}$ (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 3A** and **3B**, Section 3-3.4.2).

C (%)	Concentration of the substance under study in air
f_{evap}	fraction of evaporated substance

Inhalation exposure after spraying can be calculated by assuming instant release in a defined box (1-Box model) or a 2-Box model, according to the following (for calculations for the 1-Box model a_{inh-2} is zero):

By using a 2-Box model, the SED_{inh} can be calculated according to the equations **(7-9)** below (adapted from Rothe *et al.,* 2011). For possible parameterisation see **Appendix 11**.

$$SED_{inh} = (a_{inh-1} + a_{inh-2}) \times f_{ret} \times f_{resp} \times f_{appl}/bw \qquad \textbf{(7)}$$

$$a_{inh-1,inh-2} = a_{expo} \times r_{inh} \times t_{1,2} / V_{1,2} \qquad \textbf{(8)}$$

$$a_{expo} = a_{product} \times C_{product} \times f_{air} \qquad \textbf{(9)}$$

With:

SED_{inh} (mg/kg bw/d)	systemic exposure dose from inhalation exposure
$a_{inh-1,\ inh-2}$ (mg)	potential substance amount inhaled during boxes 1 or 2 with $V_{1,2}$, resp.
f_{ret}	fraction of substance retention in the lung (inhaled – exhaled)
f_{resp}	respirable fraction (different for pump and propellant driven aerosol sprays)
f_{appl} (day^{-1})	frequency of application
bw (kg bw)	bodyweight
$t_{1,2}$ (min)	duration of exposure in Box 1 or 2, respectively
$V_{1,2}$ (L)	volume of Box 1 or 2, respectively
a_{expo} (mg)	amount of substance available for inhalation
r_{inh} (L/min)	inhalation rate
$a_{product}$ (g)	sprayed amount of product
$C_{product}$ (mg/g)	concentration of substance in the product
f_{air}	airborne fraction

For the calculation of the substance amount, the effective concentration of substance in the product should be used by treating the propellant gas as part of the product. Otherwise, the propellant fraction can be accounted for as proposed above (Rothe *et al*., 2011).

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.

3-3.5.6 HUMAN BIOMONITORING

In most risk assessment frameworks for chemicals, the default approach to assess exposure is to calculate it from intake of exposure sources by considering single or multiple routes of exposure ('forward exposure modelling'). Often, not all possible sources and routes are aggregated, but *e.g*. only sources that are regulated under the same legislation (*e.g.* food, or cosmetics legislations). 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) in combination with a valid PBPK model ('backward exposure modelling') can be used to calculate the 'total' exposure to chemicals *via* different routes (lung, skin, digestive tract). HBM is therefore an important tool to survey the real-life internal exposure of humans and can provide more accurate data on actual internal exposure than forward exposure modelling. Therefore, 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).

3-3.5.6.1 DEFINITION

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; Polcher *et al.,* 2020).
Different biomarkers can be monitored: biomarkers that indicate exposure are called "biomarkers of exposure" (*e.g.* levels of chemical substances), whereas biological effects can be monitored by "biomarkers of effect" (*e.g.* cholesterol).

3-3.5.6.2 FIELDS OF APPLICATION FOR COSMETICS

For cosmetic ingredients, the risk of systemic effects is largely determined by skin absorption, which can be measured *in vitro* (OECD TG 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 molecules that are biologically active at low doses. In that situation, studies measuring the unchanged compound or its metabolite in urine or blood of volunteers may be valuable.
For aggregate exposure, biomonitoring data may be useful to estimate the internal dose of exposure resulting from all sources and routes of exposure (CMRs, Section 3-6.5).

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).

However, with HBM alone, it is difficult to determine the contribution of the exposure to a substance in a cosmetic product to the overall measured internal dose of exposure when other (non-cosmetic) sources for uptake and exposure also contribute considerably to the overall exposure. In such a case, HBM data and aggregate exposure modelling could support each other in risk assessment. Aggregate exposure modelling serves to determine the relative contribution of a product to the overall exposure, whereas HBM serves to evaluate whether the model over- or underestimates the real exposure.

For ingredients for which cosmetic exposure is a significant source of exposure, HBM data can be used to support the internal exposure estimation. For example, for the UV-filter benzophenone-3 (BP-3) (SCCS/1625/20), a comparison between the "external" approach as currently used by SCCS and the "internal" approach using HBM data was made (Rousselle *et al.,* 2022). Both approaches have benefits and limitations that are reflected in **Table 6.**

Table 6: Differences between current SCCS and HBM approaches for risk assessment.

	Current SCCS Approach	**HBM Approach**
Dose estimation	Modeled/estimated	Measured, real-world conditions
Exposure pathways	Dermal exposure	Provides data on total exposure from all exposure pathways
Temporality	No time lag; can be used for any substance (incl. for prediction of exposure)	Time lag between exposure estimate and risk assessment; only for substances already on the market
Product specificity	Calculations per product type, combining several conservative parameters in a deterministic assessment may lead to overestimation	No product-specific data: aggregate exposure modeling needed to identify relative contribution of a product to the overall exposure; alternatively controlled studies with selected products
Consideration for toxicokinetic aspects	Generally, uses *in vitro* studies for dermal absorption and historic animal studies for PoD and applies an assessment factor to correct for animal–human differences	Considers biotransformation and elimination of the substance in humans, but requires appropriate timing of sampling and an appropriate PBPK model
Conclusion of risk assessment for BP-3	Exposure at the intended use levels exceeds safe dose for whole-body cream and spray but not face or hand cream	Exposure exceeds safe dose in highly exposed individuals

This comparison indicates that HBM data can be useful in supporting risk assessment by providing real-life data on exposure and may also play an important role in post-approval assessment studies on exposure trends. However, before being adopted for use on a regular basis in regulatory risk assessments, more effort is needed to better harmonise HBM surveys and to obtain robust data that are representative of the exposure of the European population.

HBM data may also provide insight into the biotransformation and elimination of the substance in humans, *i.e.* toxicokinetic aspects, that with the ban of animal studies will be increasingly difficult to document. 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 that 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 support risk assessment by providing *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. If sufficient experimental animal data are available, interspecies variation between animals and humans can also be addressed.

3-4 RELEVANT TOXICOLOGICAL TOOLS FOR THE SAFETY EVALUATION OF COSMETIC INGREDIENTS

The SCCS has been closely following the progress made regarding the development and validation of alternative methods and regularly updates its NoG taking progress into consideration.
Besides validated alternatives, the SCCS may also accept, on a case-by-case basis, 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 a sufficient amount of experimental data proving relevance and reliability and 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 (GLP) laid down in 87/18/EEC (Council Directive). All possible deviations from this set of rules should be explained and scientifically justified (SCCNFP/0633/02).

3-4.1 New Approach Methodology (NAM) and Next-Generation Risk Assessment (NGRA)

3-4.1.1 DEFINITIONS

Whereas the terminology of "Alternative Test Methods (ATMs)" does not cover all available tools *e.g.*, *in silico* methodology, the more general term, NAM, has been introduced. As for cosmetics and their ingredients, testing and marketing bans apply with respect to animal use and there is an obligation to only use validated replacement alternatives, which is why.the need for validated non-animal alternative methods for chemical hazard assessment is much greater in Europe for compliance with the Cosmetics Regulation than for other regulatory frameworks. NAMs may include *in vitro, ex vivo, in chemico and in silico* methods, grouping, RAx, as well as combinations thereof. Therefore, before any testing is carried out for safety evaluation, all information on the substance under consideration should be gathered from different available means.

A set of criteria, universal across initiatives, to evaluate NAMs fit-for-purpose was developed by a multi-stakeholder group and may support greater consistency across different initiatives (Parish *et al.,* 2020).

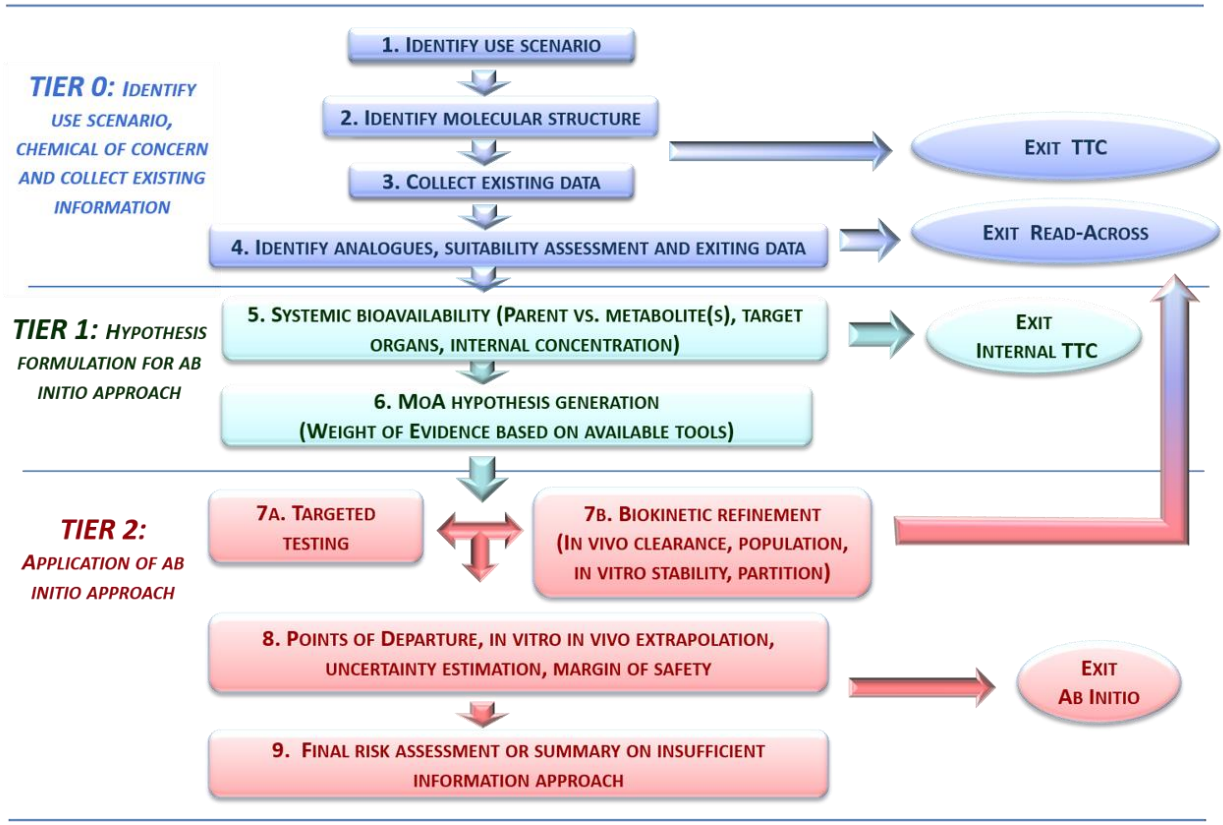
The traditional framework of risk assessment used for cosmetic ingredients is based on 3 pillars: hazard identification, dose assessment and exposure assessment (see 3-1). NAMs are in particular applied in hazard identification. They may represent stand-alone methodology and as such replace an *in vivo* method (one by one) for a specific toxicological endpoint. They may also be used in combination, *e.g.* as Integrated Approaches to Testing and Assessment (IATA) and Defined Approaches (DAs). In particular, NAMs for local (acute) toxicity became available and have been validated for regulatory purposes. However, a serious obstacle remains the lack of NAMs for systemic and long-term toxicity. Many efforts are ongoing to modernise toxicological safety evaluation and to look for non-animal methodology that can be used, not only for hazard assessment, but also for quantitative risk assessment of compounds that after long-term exposure could be at the origin of systemic toxicity. The whole traditional framework for risk assessment is under

question and one of the new approaches is the exposure-based framework, referred to as NGRA (USEPA, 2014). The principles underpinning the application of an NGRA to cosmetics have been defined by the International Cooperation on Cosmetics Regulation (ICCR), a platform of regulators and cosmetics industry from the EU, the US, Japan, Canada and Brazil. It is as follows: NGRA is a human-relevant, exposure-led, hypothesis-driven risk assessment designed to prevent harm. It integrates several NAMs to deliver safety decisions relevant to human health without the use of experimental animals. NGRA should be conducted using a tiered and iterative approach, following an appropriate literature search and evaluation of the available data, and using robust and relevant methods and strategies. It is important that the assessment should be transparently documented and explicit about the logic of the approach and sources of uncertainty (Dent *et al.*, 2018).

3.4.1.2 NGRA WORKFLOW

A general NGRA workflow is described in **Figure 6** (Berggren *et al.*, 2017).

Figure 6. Framework of the New Generation Risk Assessment (NGRA) (adopted from Berggren *et al.,*2017 and Dent *et al.,* 2018). TTC: Threshold of Toxicological Concern; MoA: Mode of Action. Copyright from Elsevier, first published in Computational Toxicology, 4, 2017.



In Tier 0, all possible information (*e.g.* external exposure, *in silico* predictions of skin absorption and metabolism) is collected (Ellison *et al.,* 2020). In particular, the so-called ADME Toolbox could be useful, not only to obtain physico-chemical properties, but also for the determination of skin and gut absorption using pig or human skin for skin absorption (Hewitt *et al.,* 2022) and Caco-2 cells for gut absorption, respectively. For distribution, parameters such as plasma stability, plasma protein binding and blood plasma/ratio are of interest (Lester *et al.,* 2021).

For metabolism, metabolic stability is important, which could be measured using, for example, S9 mix, human keratinocytes, hepatocytes, HepaRG cells (Géniès *et al.,* 2019a, 2019b; Eilstein *et al.,* 2020; Tao *et al*., 2021). Also, kidney clearance could be added to address internal exposure (Najjar *et al.,* 2022).

Tools that could be useful if NGRA would be taken as a possible workflow are described in chapters 3-4.2 to 3-4.14. **TTC** and **iTTC** approaches as risk assessment tools are taken up in 3-5.2. They preferably should not be used as stand-alone methodology, but several lines of evidence should be used in a WoE approach to come to robust conclusions.

3.4.1.3 EXAMPLES AND FURTHER DEVELOPMENTS

In the SCCS methodology workshop 2019, the use of NAMs for the safety evaluation of cosmetic ingredients was discussed to progress from concept to the practical use of NGRA with focus on systemic toxicity (Rogiers *et al.,* 2020). Several case studies were presented showing the practical feasibility of conducting NGRA for systemic effects of cosmetic ingredients *e.g.* coumarin in face cream and body lotion (Baltazar *et al.,* 2020), highlighting some critical aspects such as the need for sufficient biological coverage in terms of the mechanisms of action and cell types used, and the presence of a clear tiered workflow. Physiologically based kinetic (PBK) modelling and characterisation of some stress pathways involved were hereby applied (Moxon *et al.*, 2020; Hatherell *et al.,* 2020). Other examples given were parabens (Ouedraogo *et al.*, 2022) and the hair dye 2-methyl-1,4-benzenediamine (Goebel *et al.,* 2014).

In the meantime, more examples became available (internal Cosmetics Europe Workshop, Brussels, 13/10/2022):

- TTC applied for: Trifolium pratense (plant extract), basic blue 124 (hair dye), perillylalcohol (precursor of limonene) and chlorhexidine (antiseptic agent) (Bury *et al.,*2021).
- Grouping and read-across (RAx), supported by NAMs, for: propylparaben, genistein, daidzein, 2-ethylhexylsalicylate, avobenzone, benzoic acid & salts and esters, homosalate and caffeine (OECD GD 321).
- A 10-step framework for safety assessment: by combining RAx and NAMs (Ouedraogo et al., 2022).
- An *ab initio* approach for: phenoxyethanol (OECD GD 349), butylated hydroxytoluene, climbazole, butyl benzyl salicylate, octocrylene, ethylhexyl methoxycinnamate, benzophenone-3, benzophenone-4.

In the publications by Middleton *et al.* (2022) and Carmichael *et al.* (2022), reference is made to several substances.

A comprehensive overview of different activities in the field of NGRA has been published by Carmichael *et al*. (2022). One of these is APCRA (Accelerating the Pace of Chemical Risk Assessment), an agency-only activity in which several agencies (EPA, Health Canada, ECHA, EFSA, JRC) engage in the development of new hazard, exposure and risk assessments for their own chemical evaluation activities. Also recently, the use of a core NAM toolbox and workflow for conducting systemic safety assessments was proposed. This included PBK models and 3 bioactivity platforms (high-throughput transcriptomics, a cell stress panel, *in vitro* pharmacological profiling), from which PoDs were estimated. The protective properties for 10 compounds were determined by benchmarking against historical safety decisions. The promising outcome needs now to be confirmed with more compounds (Middleton *et al.,* 2022).
As NGRA for cosmetic ingredients does not predict toxicity thresholds, but rather looks for a safe concentration of an ingredient in a particular product, the question of how to prevent off-target toxicity is relevant. Therefore, "secondary pharmacology", as used by the pharmaceutical industry in early drug development of lead compounds, using a relatively limited panel including transporters, ion channels, enzymes, nuclear receptors, etc., was

explored by the cosmetic industry for cosmetic ingredients. It could open additional ways to create trust in the NGRA approach of safety evaluation (Burbank *et al.* 2022).

In this context, an interesting parameter has been introduced in the more recent work on NGRA, namely the bioactivity/exposure ratio = **BER**, which indicates whether use of an ingredient is safe or not and the new tools provide protection (Health Canada, 2021).

When BER is large, it is unlikely that internal levels trigger bioactivity, so there is no likelihood of adverse effects. On the contrary, when BER is small, the compound is potentially unsafe and could be rejected or additional and/or refined methodology could be necessary.

A number of case studies outside the cosmetic field, in which NGRA was applied, have been published. Examples are the assessment of genomic damage of substances in general (Dearfield *et al.*, 2017); hazard characterisation of the triazole fungicides (Van der Ven *et al*., 2020) and the industrial chemical benzene (Luijten *et al.,* 2020).

3-4.2 Adverse Outcome Pathway (AOP)

An **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 that 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/). Guidance documents OECD GD 184, GD 233, GD344 provide detailed guidance on how to document, present and assess the relevance and adequacy of an AOP. The AOP concept has been applied to a number of human-relevant toxicological endpoints including skin sensitisation (OECD GD 168) (see Section 3-4.7). The quantitative aspect is, however, still a weak point or even absent.

AOPs can be used to support the development of Integrated Approaches to Testing and Assessment (IATA) and Defined Approaches (DA) (guidance documents OECD GD 184, GD 255, GD 256, GD 260; Tollefsen *et al.*, 2014). Guidance document OECD GD 344 provides guidance on the quality standards necessary for the scientific review of an AOP on the AOP-Wiki. Furthermore, core principles associated with AOP scientific reviews are defined, thus enabling consistency in the conduction of scientific reviews, regardless of authors involved, and consequently facilitating OECD endorsement.

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 (Tollefsen *et al.*, 2014; Patlewicz *et al.*, 2015). 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 (Tollefsen *et al.*, 2014; Delrue *et al.*, 2016: Sakuratani *et al.,* 2018). Guidance document OECD GD 329 gives an overview of existing guidance on IATA and their component parts (information sources) (www.oecd.org/chemicalsafety/risk-assessment/iata/)

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 GD 255, GD 260).

3-4.3 In silico Assessment of Toxicological Hazard

In the absence of a recourse to *in vivo* testing, various *in silico* methods can offer a rapid, cost-effective, and ethical approach for estimating the toxicological hazard of a cosmetic ingredient. 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 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. 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. 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).
A number of toxicity expert systems are also available that are based on a combination of structure-activity rules, structural alerts, and/or (Q)SAR models (see below). A number of *in silico* models and tools is currently available that cover a wide variety of chemical types and many of the key toxicological endpoints that are required for risk assessment of chemical substances. Out of these, those that fulfil the quality and reliability criteria, as set out by the OECD GD 203, can be considered for use in regulatory hazard/risk assessment.

3-4.3.1 *IN SILICO* TOXICITY MODELS

(i) Quality criteria for regulatory purposes:

The toxicity estimates derived from a non-testing approach, such as a (Q)SAR model, can only be as 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 depending on 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 have limited application. 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 OECD (2004). 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 unambiguous 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.

(ii) Examples:

A few such models/systems are available in the form of both commercial and free-access software platforms that may be considered for use in regulatory hazard/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). More recently, the CONCERT REACH LIFE project updated the list of *in silico* models and tools: (https://www.life-concertreach.eu/results/results-gateway/).

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[6] 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 RAx (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 the 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.vegahub.eu). The Joint Research Centre (JRC) maintains an inventory of available QSAR models (https://eurl-ecvam.jrc.ec.europa.eu/databases/jrc-qsar-model-database).

Recently, a single software platform wrapping multiple models for cosmetics has been made available (https://www.life-vermeer.eu/download-software).

In silico models can provide estimates for dermal absorption (see 3-3.5.1.1).

(iii) Safety/hazard evaluation using *in silico* models:

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 ICCR has reviewed the use of *in silico* methods for safety evaluation of cosmetic ingredients. 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 the 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). However, despite the limitations in regard to official validation of *in silico* methods, some of the currently available high-quality models and tools can provide additional supporting evidence that can be used as part of the WoE risk assessment of cosmetic ingredients. The outcome of *in silico* assessment can also provide useful insights to help identify a toxicological hazard that can further guide the planning of more focused further (*in vitro*) testing. Indeed, the more advanced QSAR models provide not only the predicted value, but also reasoning about the potential toxic mechanism and the identity of similar substances to be used for RAx.

[6] 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.

3-4.3.2 READ-ACROSS (RAx)

(i) RAx methods and their use:

RAx methods allow derivation of toxicity estimates for an untested (target) substance from the existing data on other structurally and/or mechanistically similar (source) compounds. The fundamental tenet of RAx is that structurally similar chemicals can be expected to elicit similar effects. The method is used either in an analogue approach that involves a target and a source substance, or a category approach that involves two or more source substances. In either case, RAx allows interpolation and/or extrapolation from the available data on source substance(s) to predict toxicity of the target substance.

The process of RAx generally starts with the use of a target chemical structure to search chemical databases for source substances that are analogous to the target substance on the basis of structural similarity. However, 'similarity' is a comparative term that cannot be defined by a single metric - such as structural-similarity - and it has been recommended by ECHA and other regulatory authorities that other perspectives, such as similarity in relation to physicochemical properties, TK/TD behaviour and toxicological effects, should also be considered when selecting source analogues for use in a read-across (ECHA, 2017a).

(ii) Available tools:

It needs to be emphasised that RAx 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. The most crucial prerequisite for a reliable RAx is the identification and selection of closely similar/analogous source substances to the target substance. For the outcome of a RAx to be reliable, therefore, the database(s) used need to be of high quality and sufficiently large to contain analogues that belong to the same type/class and/or the mode of action to the target substance. The *in silico* tool/system used also needs to be transparent in terms of searching the database for the analogues.

A number of computational tools are available that can automatically find 'similar' analogues to the target substance on the basis of one, a few, or all of the aspects together (https://echa.europa.eu/en/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across).

A number of *in silico* programmes havebeen designed specifically for RAx (Patlewicz *et al.,* 2017). Since a chemical structure can be defined in terms of numerous measured and calculated physicochemical descriptors, several algorithms can be used to measure similarity between two or more compounds. Amongst the most notable ones is the k-nearest neighbour (KNN) algorithm. QSAR models based on kNN algorithm are not only useful for predicting toxicity of a substance, but they also provide a means for the identification of the closest analogues (the nearest neighbours). Similarity can be also assessed with neural network based systems - some of which are related to kNN. Other examples of *in silico* platforms that incorporate kNN based models include VEGA (www.vegahub.eu) and T.E.S.T (www.epa.gov/chemical-research/toxicity-estimation-software-tool-test).

Some *in silico* systems combine the structural similarity assessed through kNN with toxicological information and physicochemical properties. As an example, ToxEraser, implemented within VEGA platform, integrates different kinds of kNN that are based on searching for most similar compounds on the basis of structural similarity and similarities in terms of toxicological profile represented by the fragments associated with the adverse effect(s), or structural alert(s). Because such kNN-based models assign the 'neighbourhood' on the basis of all the physicochemical descriptors and the toxicological information that are used by the model, they should, in theory, provide a more comprehensive conclusion on similarity than that drawn through consideration of just one or two aspects.

The OECD toolbox provides a means for RAx from its comprehensive databases and/or additional datasets that can be added by the users. Similarly, AMBIT (http://cefic-lri.org/toolbox/ambit/) and Toxmatch (Toxmatch download | SourceForge.net) also provide useful means for identifying similar substances and read-across.

Another example is ToxRead (www.vegahub.eu/download/toxread-download/) that also displays chemical analogues in a graphic format and provides the reasoning for relevance of the effect to the target compound and a description of the statistical importance of each rule.

(iii) Special requirements:

It needs to be remembered that, unlike (Q)SAR modelling where larger datasets are required to develop better predictive models, only a few but most closely-similar analogues are generally sufficient for RAx. However, all analogues that are found within the generally accepted level for similarity (≥70% match), should be analysed and documented and justification for exclusion of any of the analogues from RAx (e.g. due to a structural or mechanistic anomaly) should be provided.

Expert opinion is needed to finally evaluate the analogues selected for RAx because certain small difference in chemical moieties, or the same moieties in the chemical structures but at different positions, may also impart a change in TK/TD behaviour and toxicological effects in two close structural analogues. This is exemplified in the case of dihydroxy and trihydroxy benzenes, in which the position of the hydroxy group(s) on the benzene ring appears to determine potential CMR properties. This highlights the crucial need for human experts to finally select, evaluate and use the analogue that may have been identified by an *in silico* system.

(iv) SCCS guidance:

Whilst *in silico* models and RAx methods provide a useful non-testing means for deriving estimates of toxicity of untested compounds, each model can have certain limitations that can impact the reliability of the results, especially when assessing different chemical types and toxicological endpoints. Therefore, the SCCS considers that the use of a single *in silico* model/system in this regard is not adequate and recommends the use of more than one relevant model/system to increase the reliability of the derived toxicity estimates. Wherever possible, a combination of *in chemico* (e.g. grouping and other chemical analogy approaches), *in silico* (e.g. QSAR models) and RAx methods should be applied to derive estimates of toxicity before experimental testing is considered. In the view of the SCCS, the toxicity estimates derived from *in silico* models/RAx alone will not be sufficient to support safety of a regulated cosmetic ingredient.

The results of *in silico* toxicity assessment are more useful for hazard assessment when they are integrated with other sources of evidence (e.g. *in vitro* results) into an overall WoE (SCCS/1578/16; EFSA, 2017a). It should also be appreciated that the use of *in silico* models and tools, and interpretation of the results, requires expert judgement, appropriate documentation and justification, and therefore cannot be accepted as the outcome of a 'black box' technology.

3-4.4 Acute toxicity

The term **acute toxicity** means those adverse effects occurring following oral or dermal administration of a single dose of a substance, or multiple doses given within 24 hours, or an
inhalation exposure of 4 hours (Regulation (EC) No 1272/2008).

In 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 use. A WoE approach may be sufficient - such as justified conclusions from

chemical grouping/read-across, (Q)SAR, *in vitro* studies, or when accessible, repeated dose toxicity studies.
Relative non-testing information sources on acute toxicity such as available approaches, (Q)SAR software packages and a list of databases from where acute toxicity data may be retrieved (ECHA 2017).
Some generic alternative approaches, mostly referring to RAx and physico-chemical properties, are presented in (OECD GD 237).

If data on acute toxicity *in vivo* are available, these data should be provided. It should be noted, however, that safety evaluation will be based on (sub)chronic toxicity studies.

3-4.4.1 ACUTE ORAL TOXICITY

A. NAMs

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). EURL ECVAM has issued recommendations concerning the validity and limitations of this *in vitro* test (EURL ECVAM, 2013). An OECD acute toxicity waiver guidance document (OECD GD 237) includes, among other criteria, the possibility to waive the acute oral toxicity study based on the results of an alternative test or test battery, if the LD50 is predicted to be greater than 2000 mg/kg.

B. *In vivo* methods

The data derived from tests discussed below should comply with the conditions mentioned in Section 1, Introduction.

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 (Regulation (EC) N° 1272/2008EC).

The original test method (EC B.1, OECD TG 401) has been replaced by alternative methods. These are still 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 TG 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 TG 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 TG 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 TG 402 for the **fixed dose procedure** is more in line with the 3R's principles. Still, draft OECD TG 434 "Acute Dermal Toxicity, Fixed Dose Procedure" (under drafting) uses fewer animals and causes less suffering.

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 TG 403). The latter was revised in 2009 (OECD TG 403, EC B.2). Furthermore, a reduction and refinement method (EC B.52, OECD TG 436), describes the **acute toxic class** method by the inhalation route. OECD TG 433 is a guideline of the **fixed concentration procedure** by inhalation. OECD GD39, provides additional information.
Research efforts have been focused on developing non-animal, human-relevant models, with emphasis on the creation of advanced *in silico* and *in vitro* models (Clippinger *et al.,* 2018a) aligned to adverse outcome pathways (AOPs) with potential relevance to toxicity following acute inhalation exposure (*e.g.* AOP 173, AOP 302) (Clippinger *et al.,* 2018b; Halappanavar *et al.,* 2020). This methodology is much more advanced than models for a repeated dose endpoint. EPA recognised the value of an alternative approach based on an *in vitro* model of the human lung epithelium (the MucilAir™ model) to refine inhalation risk assessment for the pesticide chlorothalonil, as well as for other contact irritants (US EPA, 2019). However, more work is required to gain regulatory acceptance of the *in vitro* alternatives as stand-alone replacements for animal-based acute inhalation toxicity studies, and the issues associated with interspecies differences remain unsolved.
Especially for lung adverse effects, the so-called air liquid interface (ALI) models might be useful in which the presence of the air-facing surface allows conducting *in vitro* exposures that mimic human respiratory exposures (Braakhuis *et al.,* 2020, Cao *et al.,* 2021, Petersen *et al.,* 2021a, Camassa *et al.,* 2022). The major advantage is that the cells are exposed in an air flow in a similar manner as they are in the lung, as the cells are not in a submerged culture system. Although not yet validated, these models might be especially useful for determining the possible uptake of substances, including (nano)particles from the air.

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 TG 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 (EU B.40/OECD TG 430).

2)The Reconstructed human Epidermis (RhE) Test Method includes validated commercialised human skin models *i.e.* EpiSkin™, EpiDerm™ SCT (EPI-200), SkinEthic™RHE, epiCS® (former Epidermal skin test 1000) and LabCyte EPI-MODEL24. They all consist of reconstructed human epidermal equivalent and use cell viability as an

endpoint (EC B.40bis/OECD TG 431). Only the EpiSkin™ and EpiDerm™ models are included in EC B.40bis.

3)The *In vitro* Membrane Barrier Test Method (OECD TG 435), including the Corrositex® test method.

B. *In vivo* methods:

The data derived from tests discussed below should comply with the conditions mentioned in Section 1, Introduction.

The OECD TG 404 test is no longer allowed for cosmetics and their ingredients. Data obtained from the *in vivo* skin corrosion/dermal irritation test should only be provided if the data were already available for a test performed before the animal testing ban or obtained for the purpose of demonstrating 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 TG 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 TG 439) includes a number of commercially available *in vitro* Skin Irritation Tests (SITs) that have been validated for use as:

- a stand-alone replacement test for *in vivo* skin irritation testing, or as
- a partial replacement test, within a tiered testing strategy.

Existing models are: EpiSkin™, EpiDerm ™ SIT (EPI-200), SkinEthic™ RHE and LabCyte EPI-MODEL24SIT, EpiCS, Skin$^{+®}$, KeraSkin™. Only the first four RhE models are included in EC B.46.
The endpoint used in the RhE test method is the 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 TG 431 and TG 439 support this methodology.
OECD has developed a Guidance Document GD 203 on an IATA for skin corrosion and irritation. It has several aims: to propose an integrated approach for replacing the strategy provided in the *in vivo* test guideline (OECD TG 404) and, 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* methods:

The data derived from tests discussed below should comply with the conditions mentioned in Section 1, Introduction.

The OECD TG 404 test is no longer allowed 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 of demonstrating 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.
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 (Regulation EU 2016/918).

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 eight OECD *in vitro* test guidelines adopted, which are subdivided in 4 groups (a, b, c, d). These are:

(a) *Organotypic test methods*, including two OECD guidelines:

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 TG 437). In 2020, TG 437 was updated to allow the use of a LaserLight-Based Opacitometer (LLBO) next to the standard OP-KIT device that was used for opacity measurements in the validation of the BCOP test method. The list of proficiency substances has also been updated.

2) The Isolated Chicken Eye (ICE) test method evaluates the ability of a test chemical to induce toxicity in an enucleated chicken eye (EU B.48; OECD TG 438). Since the revision of TG 438 (2018), histopathological observations may also be used as an additional endpoint to improve the prediction of some specific products, *i.e.* non-extreme pH (2<pH<11.5) detergents and surfactants. A modified version of the decision criteria for chemicals requiring classification for eye hazard has also been included.

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, 2021) definitions.

(ii) Chemicals that do not require classification for eye irritation or serious eye damage No Category according to UN GHS (2021) 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 OECD guidelines. These may be useful in providing supportive evidence (JRC 2019-2022).

(b) *Cytotoxicity and cell function-based in vitro tests*, including two 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 (EU B68/OECD TG 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 (No Category). The STE test method is not suitable for test chemicals that are insoluble or cannot be uniformly suspended for at least 5 minutes in physiological saline,

5% DMSO in saline, or mineral oil. The STE test method has limitation with respect to solid chemicals other than surfactants when used to identify test chemicals not requiring classification for eye irritation and serious eye damage (No Category).

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 TG 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).

For the Cytosensor Microphysiometer (CM) test method, the regulatory acceptance procedure has been discontinued because of lower priority.

(c) ***Reconstructed human tissue (RhT)-based test methods***, including three OECD guidelines:

5) The Reconstructed Human Cornea-like Epithelium (RhCE) test method (EU B.69/OECD TG 492) 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, four validated eye irritation test (EIT) methods using commercially available RhCE models have been adopted: the EpiOcular™ EIT, the SkinEthic™ Human Corneal Epithelium (HCE) EIT, the LabCyte CORNEA-MODEL 24 EIT and the MCTT HCE™ EIT.

6) Recently, the SkinEthic™ HCE Time-to-Toxicity test method (SkinEthic™ HCE TTT, OECD TG 492B) has been adopted as **stand-alone** method to distinguish between chemicals (substances and mixtures) that do not require classification for serious eye damage/eye irritation (No Cat.) from chemicals that require classification for eye irritation (Cat. 2), and serious eye damage (Cat. 1) and was recommended as a full replacement of the *in vivo*Draize acute eye irritation test (EU B.5/OECD TG 405). Two protocols are available, i.e. one for liquids (SkinEthic™ HCE TTL) based on the viability observed for three different exposure time periods (5, 16 and 120 min) and one for solids (SkinEthic™ HCE TTS) based on two exposure times (30 and 120 min).

7) The Vitrigel-EIT method (OECD TG 494) is an *in vitro* assay using a hCE model fabricated in a Collagen Vitrigel Membrane (CVM) chamber. The eye irritation potential of the test chemical is predicted by analysing the ability of the chemical to induce damage to the barrier function of the hCE model by measuring relative changes in TransEpithelial Electrical Resistance (TEER) over time. The Vitrigel-EIT method can be used to identify chemicals that do not require classification and labelling for eye irritation or serious eye damage within the limited applicability domain of test chemicals with a pH of > 5.0 (based on 2.5% weight/volume (w/v) preparation).

(d) ***In vitro macromolecular test method***, including one OECD guideline:

8) The Ocular Irritection (OI®) assay (OECD TG 496) is an acellular biochemical assay that evaluates the ocular hazard effects of test chemicals based on the premise that eye irritation and corneal opacity after exposure to irritating substances is the result of perturbation or denaturation of corneal proteins. The OI assay is recommended as part of a tiered testing strategy for solid and liquid chemicals under certain circumstances and with specific limitations (*i.e.*, applicable to solid and liquid chemicals whose 10% solution dispersion (v/v or w/v as appropriate) has a pH in the range $4 \leq pH \leq 9$.

An IATA for the identification of serious eye damage and eye irritation is available (OECD GD 263). Recently, a DA was also adopted at the OECD level (OECD TG 467) to provide information on potential eye hazard effects on the whole range of classifications required by the UN GHS i.e., Cat. 1, Cat. 2 and No Cat, and is thus **a stand-alone test.** Here, a fixed data interpretation procedure (DIP) (i.e. a mathematical model, a rule-based approach) is applied to a combination of e.g. *in silico* predictions, *in chemico* or *in vitro* data to predict the eye hazard potential of a test chemical. As such, a prediction is obtained without the need for expert judgment, providing increased confidence over an individual stand-alone method. OECD TG 467 contains two DAs for serious eye damage/eye irritation identification, one for neat non-surfactant liquids (DAL-1) based on physicochemical properties and *in vitro* data from RhCE (OECD TG 492) and BCOP (OECD TG 437) test methods and one for non-surfactant neat liquids, liquids and solids dissolved in water (DAL-2) based on *in vitro* data from STE (OECD TG 491) and BCOP (OECD TG 437) test methods.

B. *In vivo methods*

The data derived from tests discussed below should comply with the conditions mentioned in Section 1, Introduction.The *in vivo* test (OECD TG 405) 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

(I) *In silico* tools

Several *in silico* tools (QSARs, expert systems) have been developed to predict skin sensitisation hazard and/or potency. Some are freely available, others commercially. QSARs can be used in Defined Approaches for Skin Sensitisation (DASS). ITSv1 and ITSv2, which are two DASS incorporated in OECD TG 497 (see 3-4.7 A III), use DEREK® and the OECD QSAR Toolbox, respectively. *In silico* tools have also been proposed for use together with other NAM data in IATA or the NGRA for skin sensitisation (chapter 3-4.7.2). **Table 7** provides a non-exhaustive overview of commercial and free *in silico* tools for skin sensitisation. The stringent quality criteria and the validation principles to use these *in silico* tools for skin sensitisation hazard assessment are the same as described in Chapter 3-4.3.1 *(In silico* Toxicity Models). It should be noted that the mention of any specific software does not constitute an endorsement or recommendation by the SCCS.

Table 7: Overview of *in silico* tools for skin sensitisation

Tool	Reference
Toxtree	**Enoch *et al*., 2008** **http://toxtree.sourceforge.net/skinsensitisation.html**
OECD QSAR Toolbox	www.qsartoolbox.org
VEGA	https://www.vegahub.eu/ Chaudry *et al*., 2010
CASE Ultra®	**Gealy *et al.,* 1996; Johnson *et al*., 1997; Klopman, 2005** https://www.multicase.com
Derek Nexus®	Barratt *et al.,* 1994a,b; Langton *et al.,* 2006) https://www.lhasalimited.org/products/derek-nexus.htm
TIMES-SS®	Dimitrov *et al.,* 2005 http://oasis-lmc.org/products/software/times.aspx

The prediction of skin sensitisation by *in silico* models and/or read-across can provide a useful means for deriving evidence from non-testing methods. This line of evidence, when combined with other sources of NAM data, can support a strong overall weight of evidence for use in safety assessment.

(II) *In vitro* and *in chemico* NAMs based on KEs of AOP

Over the last years, several NAMs have been developed, validated and accepted for regulatory use (OECD GD 168, Ezendam *et al.*, 2016; Hoffmann *et al.,* 2018) that address different KEs of the skin sensitisation AOP (**Figure 7**) (see introductory part of Section 3-4.3). This AOP consists of four mechanistic key events (KEs):

MIE (**KE1**) is the covalent binding of the chemical to proteins of the skin, leading to an immunogenic hapten-carrier complex in the epidermis. After this key event is triggered, two cellular events take place: keratinocyte activation (**KE2**) and dendritic cell activation (**KE3**). Dendritic cells recognise the hapten-carrier complex and mature to migrate out of the epidermis to the local lymph node. There, the dendritic cells present the small peptides of the hapten-carrier complex to the T cells, leading to T cell activation and proliferation (**KE4**). A pool of memory T cells is generated, ultimately leading to skin sensitisation (**adverse outcome**).

An overview of the NAMs for skin sensitisation that are currently included in the OECD TG 497 and/or EU test guideline programme is provided in **Table 8**. The OECD has clustered test methods that address the same KE of the AOP in one test guideline. Currently, NAMs are available that address MIE (TG 442C), KE2 (TG 442D) and KE3 (TG 442E). There are currently no NAMs available in the OECD test guideline programme that address KE4 (T cell activation and proliferation) (van Vliet *et al.*, 2018).

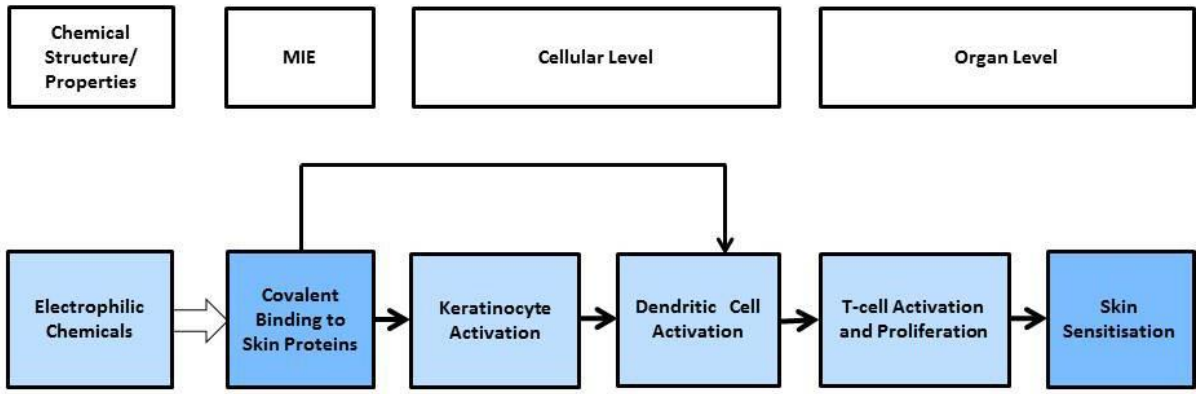


Figure 7: AOP Covalent Protein binding leading to Skin Sensitisation (taken from https://aopwiki.org/aops/40) MIE: molecular initiating event.

Besides the NAMs that are included in the OECD test guideline programme, several NAMs for skin sensitisation are still being developed or validated (Ezendam *et al.,* 2016, OECD GD 256, Hoffmann *et al.,* 2018). Two of these have been included in the work plan for the OECD Test Guidelines: the Epidermal Sensitization Assay (EpiSensA) and the SENS-IS.

- **EpiSensA** is a test method using RhE (LabCyte EPI-MODEL). Gene expression of two genes that reflect the inflammatory response and two that reflect the induction of cytoprotective gene pathways in keratinocytes is used to discriminate skin sensitisers from non sensitisers (Saito *et al.*, 2017). This NAM addresses KE2: Keratinocyte activation. The validation study of EpiSensA is currently under evaluation at the Japanese Center for the Validation of Alternative Methods (JaCVAM).
- The **SENS-IS** is a patent-protected test method using Reconstructed human Epidermis (RhE) (Episkin® RhE) together with toxicogenomic analysis for hazard and potency assessment (Cottrez *et al.,* 2015). This NAM addresses KE2: Keratinocyte activation. The validation study of this method has been evaluated by EURL-ECVAM's Scientific Advisory Committee (ESAC) (Zuang *et al.,* 2022), but this process was stopped in March 2022. The ESAC working group concluded that "they were not in the position to ensure a transparent, consistent and reliable peer review of the SENS-IS test method due to the quality of the submitted SENS-IS data". As a result, EURL ECVAM decided to stop the ESAC peer review and it is unclear if and when this test method will be further evaluated by the OECD for inclusion in their test guideline programme.

Table 8: 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 TG 442C (2022) / EC B.59 *In chemico* skin sensitisation: Assays addressing the AOP key event on covalent binding to proteins	Direct Peptide Reactivity Assay (DPRA) Amino acid Derivative Reactivity Assay (ADRA) Kinetic Direct Peptide Reactivity Assay (kDPRA)
KE2: keratinocyte activation	OECD TG 442D (2022) / EC B.60 *In vitro* Skin Sensitisation: ARE-Nrf2 Luciferase Test Method	ARE-Nrf2 Luciferase KeratinoSens™ Test Method The ARE-Nrf2 luciferase LuSens test method

KE3: dendritic cell activation	OECD TG 442E (2022) / 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) Genomic Allergen Rapid Detection (GARD™) for the detection of skin sensitisation (GARDskin™)

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

(III) *Defined approaches on skin sensitisation* (DASS)

The currently available NAMs for skin sensitisation address a single key event of the AOP and are therefore often combined in testing strategies to cover multiple key events. In addition, individual test methods have some known technical limitations, which may lead to false-negative results if the tests were used as a stand-alone method. These technical limitations are described in the OECD test guidelines. For abovementioned reasons, a single alternative method cannot be used as a stand-alone assay for hazard identification and potency sub-categorisation of skin sensitisers. It is therefore recommended to combine these methods and other information sources (*e.g.*, *in silico* tools) in an integrated approach such as a DASS or IATA. Different DASS have been proposed, some of them can only be used for hazard identification, whereas others use quantitative data to predict a potency category or a NOAEL/NESIL (Ezendam *et al.,* 2016, OECD GD 256, Kleinstreuer *et al.,* 2018).

Three DASS have been included in OECD TG 497 on DAs on Skin Sensitisation (2021). The guideline describes three DAs that have been shown to provide information that is equivalent to that provided by the Local Lymph Node Assay (LLNA).

1. The "2 out of 3" (2o3) defined approach to skin sensitisation hazard identification based on *in chemico* (KE1) and *in vitro* (KE2/KE3) data from DPRA, KeratinoSens™ and h-CLAT.
2. The integrated testing strategy (ITSv1) for UN GHS potency categorisation based on *in chemico* (KE1) and *in vitro* (KE3) data, DPRA, KeratinoSens™ and h-CLAT and *in silico* (Derek Nexus) predictions
3. A modification of the integrated testing strategy (ITSv2) for UN GHS potency categorisation based on *in chemico* (KE1) and *in vitro* (KE3) data, DPRA, KeratinoSens™ and h-CLAT and *in silico* based on *in chemico* (KE1) and *in vitro* (KE3) data, and *in silico* (OECD QSAR Toolbox) predictions.

The OECD has included two projects related to DASS in their workplan of the OECD test guideline programme:

1. Substitution of “Me-Too” Information Sources into Defined Approaches for Skin Sensitisation within OECD TG 497. The three current DASS in this guideline are based on three NAMs: DPRA, KeratinoSens™ and h-CLAT. The aim is to allow the use of the other NAMs for skin sensitisation that are included in the OECD test guidelines in the DASS.
2. Feasibility Study on the Inclusion of the Skin Allergy Risk Assessment (SARA) model into OECD TG 497 on Defined Approaches on Skin Sensitisation. This DASS makes use of a Bayesian statistical approach to calculate a human-relevant metric of sensitiser potency. The model is based upon any combination of human repeat insult patch test, local lymph node, direct peptide reactivity assay, KeratinoSens™, h-CLAT or U-SENS™ data (Gilmour *et al.,* 2022, Reynolds *et al.,* 2019, 2022).

DASS that are not yet included in OECD Guideline 497 may be submitted to the SCCS in the context of the NGRA for skin sensitisation (3-4.7.2). To be able to fully understand and evaluate the submitted DASS, it is essential that information on how the model is built is provided. The OECD has developed a reporting template that can be used for this purpose (provided as an Annex to OECD GD 256).

B. *In vivo methods*

The data derived from tests discussed below should comply with the conditions mentioned in Section 1, Introduction.
Three regulatory-accepted *in vivo* laboratory animal test methods have been used to evaluate the potential of a substance to cause skin sensitisation, the LLNA, the Magnusson Kligman Guinea Pig Maximisation Test (GPMT) and the Buehler test (**Table 9**). The GPMT and Buehler tests are able to provide results on induction and elicitation; the LLNA and its variants only address induction.

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 10**) (ECB, 2002; Basketter *et al.*, 2005).

Table 9: *In vivo* laboratory test methods for evaluation of skin sensitisation

Species	***Test method***	***Endpoint***	***Guideline***
Mouse	LLNA (radioactive method)	Cellular proliferation SI≥3	OECD TG 429, EC B.42
Mouse	LLNA:DA (non-radioactive method)	Cellular proliferation SI≥1.8	OECD TG 442A, EC B.50
Mouse	LLNA: BrdU-ELISA (non-radioactive method)	Cellular proliferation SI≥1.6	OECD TG 442B, EC B.51
Guinea pig	GPMT	Score of erythema and swelling	OECD TG 406, EC B.6
Guinea pig	Buehler test	Score of erythema and swelling	OECD TG 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 IndustrieLLNA: BrdU-ELISA: nonradioactive modification of LLNA based on cell proliferation measured by 5-Bromo-2'-deoxyUridine

Table 10: 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 (ECB, 2002; Basketter *et al.*, 2005). In the absence of LLNA data, this subcategorisation can be used as indicative for potency.

3-4.7.1 SKIN SENSITISATION QUANTITATIVE RISK ASSESSMENT (QRA)

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 that isnot expected to cause induction of sensitisation (No Expected Sensitising Induction Level or 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 (https://www.ideaproject.info/), the QRA was further refined by including aggregate exposure assessment and revising the SAFs.

A technical dossier describing the revised QRA (QRA 2) was submitted by the fragrance industry to the SCCS. After evaluation of the methodology, SCCS concluded that a lot of progress had been achieved since the initial publication of the QRA. Also, a peer-reviewed publication on the QRA2 methodology was published (Api *et al.*, 2020), summarising the progress made in this field so far. As some aspects of the methodology need further clarification, it was decided that a case study of a fragrance ingredient would made it clear whether QRA2 in its actual form can be practically applied or some changes sare sill needed. Once again, this methodology could be a useful tool not only for safety evaluation of fragrance allergens, but potentially also for other cosmetic ingredients (SCCS/1589/17).

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 (referred to in SCCS/1567/15).

Further developments:
Regarding fragrances, *in vitro* methods to obtain a measure of sensitisation potency (*i.e.* a step further than hazard assessment) are being evaluated within the IDEA project (https://www.ideaproject.info/). A Reference Chemical Potency List (RCPL) of 33 chemicals (fragrance materials and other chemicals), spanninga range of chemical and skin sensitising potency and integratingexisting EC3 data from earlier LLNAs and (if available) human tests, has been developed (Irizar *et al.,* 2022). The anticipation is that the RCPL will provide a template for evaluating the accuracy of *in vitro* methods for measuring skin sensitising potency.

3-4.7.2 NEXT-GENERATION RISK ASSESSMENT APPROACH (NGRA)

NGRA developed for systemic toxicity of cosmetic ingredients (Berggren *et al.,* 2017, see also **Fig 6** under 3-4.1) has been used as a framework for skin sensitisation safety assessment (Gilmour *et al*., 2020), taking the same principles into consideration. A tiered workflow is applied as illustrated in **Fig 8** for skin sensitisation.

For the SCCS, NGRA is a novel conceptual iterative approach that could offer the possibility of integrating existing data, RAx information and NAM information. This framework could deliver all available and newly generated data in a systematic and structured way. The SCCS needs to build up experience with the NGRA, as well as with DASS (3-4.7 B), and will evaluate and accept the approach on a case-by-case basis.
In Tier 0, exposure-based waiving, using the 'Dermal Sensitisation Threshold' (DST) concept, has been proposed as an exit. However, the SCCS is of the opinion that practical experience and the necessary confidence in reliability are still lacking.

The key aspects of NGRA for skin sensitisation can be found in the publication by Gilmour *et al*., (2020). Some case studies, in which NGRA for skin sensitisation has been applied to a cosmetic ingredient, are available (Vandecasteele *et al.,* 2021, Gautier *et al.,* 2020, Reynolds *et al.*, 2021).

To provide the SCCS with an accurate description of the DA used in the NGRA framework and to facilitate the evaluation, the SCCS recommends using the reporting format of the OECD for DAs that are not included in OECD 497 (2021), *e.g.* SCCS Opinion on Sodium Bromothymol Blue (SCCS/1645/22).

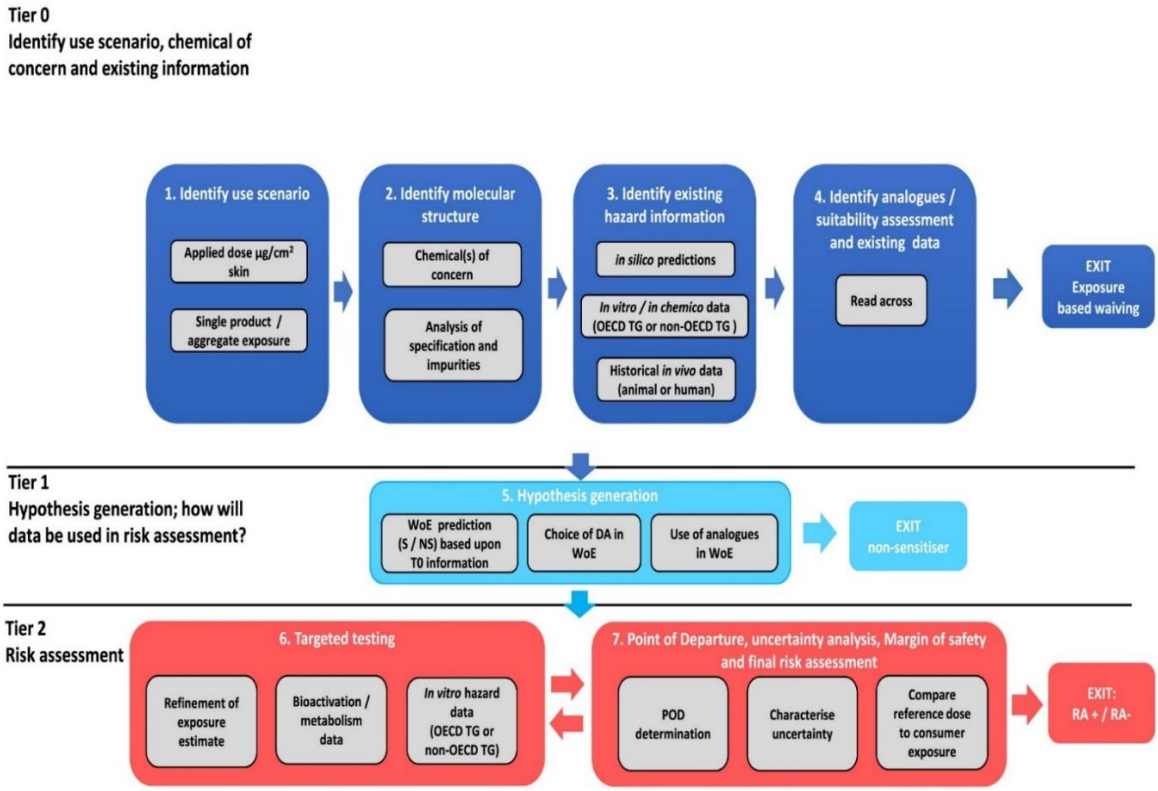


Figure 8: Framework for skin sensitisation safety assessment (Gilmour *et al.*, 2020). S/NS: safe/not safe; DA: defined approach; WoE; weight of evidence; PoD: point of departure; RA+/RA-: risk assessment positive/negative outcome. Taken from Regulatory Toxicology and Pharmacology, 2020, 116, Gilmour *et al*., with permission from Elsevier.

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. <u>NAMs</u>

No validated alternative methods are available yet for determining the repeated dose toxicity of a substance, which poses a problem for the introduction of new compounds, *e.g.* preservatives on the EU market as this assay usually provides the PoD of the compound under investigation (necessary for MoS calculation). Efforts are being made by the cosmetic industry to develop an NGRA strategy as an alternative for not having a PoD generated via *in vivo* methodology (see 3-4 and 3-4.1). The topic was extensively discussed in the February 2019
SCCS methodology workshop with the aim to go from concept to practical use with a clear focus on systemic toxicity (Rogiers *et al.,* 2020).
The progress, made since then, is taken up under 3-4.1.
Several detailed case studies are available on integrated approaches for systemic toxicity (*e.g.* OECD 321 and 349, caffeine and phenoxyethanol, respectively), but more trust still

needs to be created that NGRA and integrated approaches for systemic toxicity are protective enough for human health and that unexpected side effects are not occurring.

B. *In vivo* methods

The data derived from tests discussed below should comply with the conditions mentioned in Section 1, Introduction.

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

1)
- Sub-acute oral toxicity (28 days) (EC B.7, OECD TG 407)
- Sub-acute dermal toxicity study (28 days) (EC B.9, OECD TG 410)
- Sub-acute inhalation toxicity study (28 days) (EC B.8, OECD TG 412)

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

3)
- Chronic toxicity studies (primarily rodents) (EC B.30, OECD TG 452)
- Combined chronic toxicity/carcinogenicity studies (EC B.33, OECD TG 453) (primarily rodents)

For 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.

3-4.8.1 THE USE OF UNCERTAINTY FACTORS (UFS) FOR EXTRAPOLATION FOR STUDY DURATION

This type of UF is used to take account of probable differences between the experimental setting from which the PoD is taken and the human real-life situation (use scenario) in case substance-specific information is lacking.

For some cosmetic ingredients, dermal repeated dose toxicity studies are submitted. These studies, if of good quality, are taken into consideration by the SCCS as it is the most commonly used application route for cosmetics.

In practice, however, oral route studies are often used for the MoS calculation to consider (worst case) systemic exposure. Oral repeated dose toxicity studies can be either subacute (28 days), subchronic (90 days) or chronic (taking 85% of lifetime).

The 90-day oral toxicity test in rodents was, historically speaking, the most commonly used repeated dose toxicity test for cosmetic ingredients. Based on the exposure and the short lifetime of cosmetic products (regularly changing ingredients and concentrations), the 90-day test provides a good indication of the target organs and the type of systemic toxicity.
In case only a 28-day study is available, the SCCS recommends applying a factor to take uncertainty into consideration to extrapolate from subacute (28 days) to subchronic (90 days) toxicity**.** Different values are being proposed and the choice is made on a case-by-case basis taking the strengths and weaknesses of the available studies into consideration. The SCCS commonly uses for this type of, extrapolation a conservative **UF of 3.** Recently, Escher *et al*., (2020) provided data showing that in such a case a factor of 1.5 would be sufficient.
When a scientifically sound 90-day study is available which allows for the determination of a clear PoD, the SCCS takes this study into account for calculating the MoS. An uncertainty factor is only included when some doubt exists with respect to the quality of the subchronic toxicity study and/or in the absence of further information supporting the PoD from the 90-day study (*e.g.* availability of a chronic study). Escher *et al.,* (2020) proposed a factor of 1.5. In other domains (environmental, food, …) higher factors have been proposed, but these may contribute to a higher variance. In any case, the use of additional UFs needs to be carefully
considered. Indeed, many authors warn that a composite UF may lead to over conservatism (Simon *et al.,* 2016; Escher *et al*., 2020). In particular, in the case of aggregate exposure, using a deterministic exposure assessment multiplication of single UFs may lead to possibly overly conservative estimates (EFSA, 2012a).

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. Because of the likelihood of high uncertainty in regard to different inhalable products and their modes of delivery, the SCCS recommends analysis of uncertainty on a case-by-case basis.
When reproductive toxicity studies are used to determine the PoD, the uncertainty factors for extrapolation for study duration are not used.
In sections 3.5.1.1 and 3.5.1.2 a number of default factors are discussed.

3-4.8.2 SELECTION OF POD

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, also in the context of cosmetic exposure. For example, local effects on the gastrointestinal tract, sometimes observed with irritants after oral exposure, are not considered relevant by the SCCS to be used for the MoS calculation. A BMD, NOAEL or LOAEL is derived for each study and the most relevant study in terms of quality, duration of exposure, and available PoD is then selected by the SCCS to be used for the safety evaluation. 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 made for a period of longer use.

As part of the European Green Deal policy, a Mixture Assessment Factor (MAF) is introduced for industrial chemicals (EU Chemicals Strategy for Sustainability (COM/2020/667, final). Different definitions for MAF exist which could affect the final outcome. The SCCS closely follows up the developments, which could have important implications for cosmetics.

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 2019-JRC 2022).

Since the field of reproductive toxicity is very complex, it was expected that the various phases could not be mimicked using one alternative method and that a battery of tests would be 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 the ECVAM Scientific Advisory Committee (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 can be found in JRC reports (JRC 2019-JRC 2022). 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. *In vivo* methods

The data derived from tests discussed below should comply with the conditions mentioned in Section 1, Introduction.

The most commonly performed *in vivo* reproductive toxicity studies are:
1) Two-generation reproductive toxicity study (EC B.35, OECD TG 416)
2) Prenatal developmental toxicity study[7] - rodent and non-rodent (EC B.31, OECD TG 414)

[7] Often also named teratogenicity test

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

The Extended One-Generation Reproductive Toxicity Study (EOGRTS) has been adopted by the OECD (OECD TG 443). 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 balano-preputial separation)
- ☐ Increased statistical power with respect to parameters for reproductive toxicity
- ☐ Possibility for modification, *e.g.,* to include new endpoints for the assessment of endocrine active chemicals disrupting the Hypothalamus-Pituitary-Gonad (HPG) axis, the somatotropic axis, the retinoid signalling pathway, the Hypothalamus-Pituitary-Thyroid (HPT) axis, the vitamin D signalling pathway and the Peroxisome Proliferator-Activated Receptor (PPAR) signalling pathway

A 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 **Appendix 1, section 3** and **Appendix 4**).

3-4.10 Mutagenicity / Genotoxicity

3-4.10.1 DEFINITIONS

Mutagenicity: a mutation is defined as a permanent change in the amount or structure of the genetic material. The terms 'mutagenic' and 'mutagen' are used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms and applies both to heritable genetic changes that may be manifested at the phenotypic level and to the underlying DNA modifications (including specific base pair changes and chromosomal aberrations).
Germ cell mutations are those that occur during spermatogenesis/ovogenesis and appear in the egg or sperm (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.

Genotoxicity: the more general terms 'genotoxic' and 'genotoxicity' apply to agents or conditions that alter the structure, information content, or segregation of DNA, including those which cause DNA damage by interfering with normal replication processes, or which alter its replication in a non-physiological manner (temporarily).

3-4.10.2 MECHANISMS

There are several mechanisms that lead to genotoxicity. In general, DNA damage can arise through either primary or secondary mechanisms (Magdolenova *et al*., 2014, Evans *et al*., 2017).

Primary genotoxicity can be either direct, where there is a direct interaction of genotoxic agent with DNA, or indirect, where the genotoxic effect is exerted via intermediate molecules (such as free radicals, as in oxidative stress). Secondary genotoxicity is driven,

for example, by oxidative stress arising from inflammation caused by activation /recruitment of immune cells such as macrophages or neutrophils. Where the evidence suggests indirect mechanisms (*e.g.* oxidative stress, topoisomerase inhibition), or secondary mechanisms (*e.g*. inflammation and oxidative stress caused by inflammation via overexpression of the immune cells), a threshold may be derived from the toxicological data for use in safety assessment.

Based on recommendations of international groups of scientific experts (Dearfield *et al.*, 2011), and in accord with EFSA (EFSA, 2011a) and the UK Committee on Mutagenicity (COM, 2011 and 2020), 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 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. Genotoxicity Indicator tests, which measure DNA damage without taking into account the consequences of this primary damage, can contribute to the weight of evidence approach but should not be used as stand-alone tests. That is the case, for example, of in vitro comet assay or DNA adducts formation assay. Finally, before undertaking any testing, a thorough review should be carried out of all available data on the substance under assessment.

A. NAMs

(i) *In silico* methods for genotoxicity and carcinogenicity

- Genotoxic carcinogens (DNA reactive)

As explained in the testing strategy for mutagenicity/genotoxicity (**Figure 9**, section 3-4.10), the use of structure-activity relationship-based *in silico* models and read-across can provide a useful indication of the mutagenic/genotoxic and carcinogenic potential of a cosmetic ingredient.
The regulatory requirements for testing certain categories of chemicals have led to a large database on genotoxicity over the past decades, in particular with information on bacterial reverse mutation (Ames) tests, as well as on *in vitro* and *in vivo* micronucleus tests, and chromosomal aberration tests. As a result of this growing database, and of research on mode of action, there is a better understanding of the mechanisms of mutagenicity/genotoxicity via direct or indirect interaction of chemical s ubstances with the genetic material, compared to certain other complex toxicity endpoints.

The knowledge deciphered from the available information has indicated that the chemicals that can cause mutagenic/genotoxic effects through direct interaction with DNA are either intrinsically electrophilic, or they can be transformed to electrophilic intermediates. On the other hand, some chemicals may contain one or more structural alerts for genotoxicity but may not cause genotoxic effects because of their (higher) molecular weight, solubility, chemical reactivity, structural geometry, etc. (Plošnik *et al.,* 2016).
The OECD QSAR Toolbox incorporates a number of databases on mutagenicity /genotoxicity and carcinogenicity that provide a valuable resource for RAx.

- The toolbox on *in vitro* genotoxicity includes bacterial mutagenicity ISSSTY; ECHA REACH; OASIS genotoxicity; EFSA pesticide genotoxicity.

- The databases on *in vivo* genotoxicity include ECHA REACH; ECVAM genotoxicity and carcinogenicity; EFSA pesticide genotoxicity; ISSMIC Micronucleus; OASIS Micronucleus.

Formely developed Carcinogenic Potency Database (CPDB) containing data on substances derived from long-term carcinogenicity tests on chemicals in rats, mice, dogs, hamsters and non-human primates has been replaced by Lhasa Limited. The Lhasa Carcinogenicity Database (LCDB) contains all data from the original CPDB database and has since been supplemented with additional data

(https://www.lhasalimited.org/products/lhasa-carcinogenicity-database.htm).

- *in silico* methods (structure-activity based) for the prediction of carcinogenicity of chemical substances include the open-source tools LAZAR (https://lazar.in-silico.ch/predict) and (Q)SAR models such VEGA (https://www.vegahub.eu).

The availability of large amount of data on mutagenicity/genotoxicity and carcinogenicity has also enabled the identification of key molecular descriptors and structural alerts associated with mutagenicity/genotoxicity *(e.g.* Ashby and Tennant, 1988; Benigni and Bossa, 2008; Plošnik *et al.,* 2016), and the development of several structure-activity based *in silico* (Q)SAR models and read-across systems. A number of these systems have been developed using high quality data and were subjected to stringent assessments to verify their reliability for use in regulatory risk assessments.

- Non-genotoxic carcinogens (DNA-non reactive)

In comparison to genotoxic carcinogens, the identification of Non-Genotoxic Carcinogens (NGCs) is much more difficult because, unlike the direct or indirect interaction of genotoxic substances with DNA, the carcinogenic effects of NGCs may manifest from a variety of different mechanisms, not always relevant to humans.

NGC are thought to have a safe exposure threshold or dose; thus, their use in society is accepted provided that the exposure or intake levels do not exceed the threshold. For these reasons, the *in silico* methods for the identification of NGCs are based on a limited number of structural alerts that have so far been identified.

Examples of available *in silico* systems

[8]As already mentioned in section 3-4.2, the EU project ANTARES has listed the available free-access and commercial *in silico* models and tools; this list has been updated within the CONCERT REACH LIFE project and is available on the project website (https://www.life-concertreach.eu/results/results-gateway/).

The notable free access *in silico* systems for the assessment of mutagenicity/genotoxicity and carcinogenicity (for which more information is present in **APPENDIX 10**) include:

- The Danish QSAR database (http://qsar.food.dtu.dk/)
- The OECD QSAR Toolbox (https://qsartoolbox.org/),
- VEGA QSAR platform (https://www.vegahub.eu/)
- The US-EPA's Toxicity Estimation Software Tool (T.E.S.T.) (https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test)
- Toxtree (http://toxtree.sourceforge.net/)
- OpenTox for carcinogenicity (https://opentox.net/)
- Lazar (https://lazar.in-silico.ch/predict)
- OncoLogic (US EPA) (www.epa.gov/tsca-screening-tools/oncologictm-expert-system-evaluate-carcinogenic-potentialchemicals)

A number of **commercial systems** are also available for the assessment of potential mutagenicity/genotoxicity and carcinogenicity. These include QSAR-based systems such SciQSAR® (SciMatics, Inc.) and TopKat® (Toxicity Prediction by Komputer Assisted Technology); molecular fragment-based QSAR expert systems such as CASE-Ultra® (Multicase Inc.) and Leadscope® (Leadscope, Inc.); and expert knowledge-based systems such as Derek Nexus® (Lhasa Ltd.). In addition to these *in silico* models, RAx tools for mutagenicity, genotoxicity and carcinogenicity also are available and can find structurally

[8] The list of *in silico* models/systems is not exhaustive, and the mention of any model/system here does not constitute an approval of its quality, or recommendation for use by the SCCS.

similar substances and indicate their potential mechanism of action. Examples are ToxRead and VERA (www.vegahub.eu).

Protocols for *in silico* assessment of genetic toxicity have been described by Hasselgren *et al.*(2019) and a number of studies have assessed the reliability of the *in silico* methods for the prediction of genotoxicity and carcinogenicity. The results have generally confirmed that a number of the *in silico* systems can provide a high degree of reliability for the estimation of genotoxic potential of chemicals (Serafimova *et al.*, 2010; Bakhtyari *et al.,* 2013; https://www.life-concertreach.eu/results/results-gateway). More recently, Honma *et al.,*(2019) have tested 17 QSAR tools using a proprietary Ames mutagenicity database containing 12140 new chemicals, at least 85% of which were not included in publicly available or commercial databases and had not been used in QSAR modelling under the Ames/QSAR International Challenge Project. Their findings indicate that most tools achieved >50% sensitivity (positive prediction among all Ames positives) and predictive power (accuracy) ashigh as 80%, which is almost equivalent to the inter-laboratory reproducibility of the Ames tests. In the research conducted by EFSA (Benigni *et al.*, 2019), RAx was applied to both Ames and *in vitro* chromosomal aberrations assays, with two new strategies based on
different approaches and integrating different sets of information. A common result was that RAx appeared to be largely successful for predicting the Ames test results. The performance of the two strategies was partially different with *in vitro* chromosomal aberrations, but overall, it was lower than that obtained with the Ames test.
These assessments point to the potential of *in silico* methods and models to generate supporting evidence on the potential mutagenicity/genotoxicity of cosmetic ingredients to support the WoE on their safety in conjunction with other (*in vitro)* data. As indicated in section 3.4.2, the estimates derived from *in silico* models and RAx can provide useful additional supporting evidence for hazard assessment, especially when the results are integrated with other sources of evidence (*e.g. in vitro* data) into an overall WoE for use in risk assessment of cosmetic ingredients.

(ii) From a 3-test *in vitro* battery to a 2-test *in vitro* 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 that were used (Kirkland *et al.*, 2011). Consequently, GUM (Pfuhler *et al.*, 2007), EFSA(2011a) and COM (2011, last update 2021) 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 that 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 the *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 for measuring numerical chromosomal aberrations (Corvi *et al.,* 2008).
In line with this, the SCCS recommends two tests for the base-level testing of cosmetic substances, represented by the following test systems: (i) Bacterial Reverse Mutation Test (OECD TG 471) as a test covering gene mutations. Recently, OECD TG 471 has been revised with CAS reference numbers of strain-specific positive controls and (ii) *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. If an applicant resubmits study results because of quality issues identified by the SCCS while reviewing

the study, priority should still be given to performing studies according to the OECD accepted TG.

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, *e.g.* for the measurement of nanoparticles, or for biocidal compounds and antibiotics, a scientific justification should be given and a gene mutation test in mammalian cells, like the Hprt/Xprt (OECD TG 476) or the thymidine kinase Tk (OECD TG 490), should be performed.

EURL ECVAM developed a genotoxicity and carcinogenicity database of substances eliciting negative results in the Ames test (Madia *et al*., 2020; https://data.jrc.ec.europa.eu/dataset/38701804-bc00-43c1-8af1-fe2d5265e8d7).

When testing nanomaterials, especially with negative results, evidence is needed to show that the nanoparticles were internalised by the test system or entered in contact with DNA. For further considerations of particle-related behavior of substances, the Applicants should refer to SCCS/1611/19: Guidance on the Safety Assessment of Nanomaterials in Cosmetics.

(iii) Novel *in vitro* approaches in genotoxicity models:
The recommendations and conclusions from the International Workshops on Genotoxicity Testing (IWGT) (Martus *et al*., 2020) concerning different methods are supported by the SCCS:

1. The Ames Test:
    a. critical issues to be considered to bring OECD TG 471 up to date and make it consistent with other OECD TGs have been identified (Williams *et al*., 2019; Levy *et al*., 2019a and 2019b).
2. The Mammalian Cell Gene Mutation Assays:
    a. *In vitro* TransGenic Rodent (TGR) mutagenicity assays, once validated, could be employed for routine mutagenicity assessment, as they have endogenous metabolic capacity and consequent ability to generate DNA-reactive metabolites - properties lacking in cell lines frequently employed for *in vitro* testing (White *et al.,* 2019).
    b. *In vitro* mutagenicity assays based on immortalised cell lines or primary hepatocytes from the MutaMouse or lacZ Plasmid Mouse are at an advanced stage of validation.
    c. The Phosphatidylnositol glycan class A gene (*Pig-a*) mutagenicity assay is at an early stage in terms of safety testing and hazard identification (Dertinger *et al.,* 2021);
    d. The sensitivity of the Mammalian Cell Gene Mutation Assay can be improved by the use of XRCC1$^{-/-}$/XPA$^{-/-}$ TK6 cells (Ibrahim *et al.,* 2020).
3. Novel & Emerging *in vitro* Mammalian Cell Mutagenicity Test Systems:
    a. genome-wide loss-of-function screening, mutation characterisation by next generation sequencing, and fluorescence-based mutation detection can be promising methods (Evans *et al*., 2019a).
4. The 3D Tissues in Genotoxicity Testing (Pfuhler *et al.*, 2020a, 2020b):
    a. 3D tissue models simulate *in vivo*-like conditions regarding cell viability, proliferation, differentiation, morphology, gene and protein expression. They can complement classical 2D cell culture-based assays;

b. 3D tissue-based genotoxicity assays can be used as 2nd tier assays to follow-up on positive results from standard *in vitro* assays;
c. For adoption of a tissue model as a 2nd tier assay, ability to detect the full range of genotoxic damage (leading to mutagenicity, clastogenicity, aneugenicity) should be demonstrated;
d. The 72-hour protocol for the 3D Reconstructed human Skin MicroNucleus assay (RSMN) has higher sensitivity than the 48-hour protocol;
e. The 3D skin Comet (Pfuhler *et al.*, 2020a) and MN (Pfuhler *et al*., 2021) assays are now sufficiently validated to move towards individual OECD Test Guidelines, but an independent peer review of the validation study is still needed.

5. High Information Content assays:
   a. adductomics, global transcriptional profiling, error-reduced single-molecule sequencing, and multiplexed phenotypic profiling are promising tools for regulatory purposes (Dertinger *et al*., 2019).

(iv) *In vitro* models for secondary genotoxicity:

A significant knowledge gap exists in regard to which *in vitro* system(s) might be appropriate for assessing secondary (inflammation-driven) genotoxicity (OECD, 2014). Several *in vivo*-like *in vitro* models addressing inflammation driven genotoxicity have been developed, ranging from a simple conditioned medium approach (*e.g.* exposing THP-1 derived macrophages and then transferring the conditioned medium to bronchial cells) to more complex co-culture models (Evans *et al*., 2017, 2019b; Åkerlund *et al*., 2019). The most advanced models, comprising either two or more different cell types co-cultured with immune cells, were reviewed (Evans *et al*., 2017) and discussed during the 7th IWGT in Japan 2017 (Pfuhler *et al*., 2020b, Martus *et al*., 2020). They encompass cell-to-cell interplay, which promotes intracellular signalling and molecular crosstalk, representing more *in vivo*-like conditions.

(v) Outcome of *in vitro* tests

If the results from both tests (Bacterial Reverse Mutation Test (OECD TG 471) and the *In vitro* Micronucleus Test (OECD TG 487) are clearly negative in adequately performed tests, it is very likely that the substance has no mutagenic potential. Likewise, if the results from one or both tests are clearly positive, it is very likely that the substance has genotoxic/mutagenic potential. In both cases further testing is not necessary.
A general scheme of mutagenicity testing for cosmetic ingredients is presented in **Figure 9.**

Additional information for *in vitro* testing can be found in COM 2011.

Different and potentially contradicting results may be available from the same test when performed with non-standardised protocols and carried out by different laboratories. In such cases, expert judgement should be used to evaluate and interpret the data. Further tests may be necessary to reach an overall conclusion.
Special attention should be given for poorly soluble chemicals. The determination of solubility

in the culture medium prior to the experiment is mandatory. 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 a visible precipitate because inaccurate effects may result from the precipitate. At the concentration producing a precipitate, care should be taken to ensure that the precipitate does not interfere with the conduct of the test (*e.g.* staining or scoring).

For substances present as impurities in cosmetics, potential genotoxicity alerts should be determined using ToxTree software by applying different decision trees or with OECD QSAR ToolBox. If presence of such alerts is confirmed and the amount of the impurity is known, then Margin of Exposure (MoE) should be calculated by dividing derived SED value by the threshold for Class III substances with possible DNA reactive ability of 0.15 µg/person/d, corresponding to 2.5 ng/kg bw/d. If MoE is higher than 1, the level of the impurity can be considered of no concern.

(vi) Toolbox for further evaluation in a WoE approach

- The 3D reconstructed human skin Comet and micronucleus (RSMN) assays can support a WoE approach in the case of a positive or equivocal bacterial or mammalian gene mutation test and micronucleus/chromosomal aberration test, especially for dermally applied compounds. The experimental phase of the validation has been finalised (Pfuhler *et al.,* 2020a) and the standard project submission form (SPSF) for both assays have been accepted by OECD and included in the OECD work plan in 2019 (OECD 2021).
- Another useful tool is the Hen's Egg test for Micronucleus Induction (HET-MN), which is currently under evaluation (JRC 2019-JRC 2022; Reisinger *et al.,* 2019, 2021) and the validation dataset has just been accepted for publication (Maul *et al.,* 2022). This enables follow-up testing for systemically available compounds.
- The enzyme-linked comet assay for detection of oxidized DNA bases can be useful for identification of a genotoxicity involving oxidative stress. Standardisation and pre-validation of the method have been conducted recently by the COMET consortium (Møller *et al.,* 2020; Collins *et al.,* 2023) and the application for an OECD test guideline is in preparation.

Negative results from these alternative tests alone 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 GreenScreen HC™, BlueScreen HCTM used to screen the genotoxic and cytotoxic potential of chemicals and ToxTrackerTM (at present under formal validation, SPSF was accepted by OECD 2020 (Test Guideline Programme Work Plan 2020) 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). ToxTrackerTM was able to accurately classify compounds as genotoxic or non-genotoxic, and could discriminate between DNA-reactive compounds, aneugens and indirect genotoxicity caused, *e.g*. by oxidative stress (Brandsma *et al*., 2020, Wills *et al*., 2021, Misik *et al*., 2022).
- The results obtained using a reporter gene assay provide mechanistic information at the molecular level but cannot alone overrule a positive result from an *in vitro* batteryas the assay 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 (Magkoufopoulou *et al.,* 2012) or HepaRG™ cells (Ates *et al.,* 2018).
- The level of phosphorylated form of H2AX histone (γH2AX) in cells exposed to a chemical can indicate its potential for induction of DNA damage (Kopp *et al.,* 2019). 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. Such promising assays are MultiFlow and the Multi-Endpoint Genotoxicity Assay (MEGA-Screen system) (Dertinger *et al*., 2019).

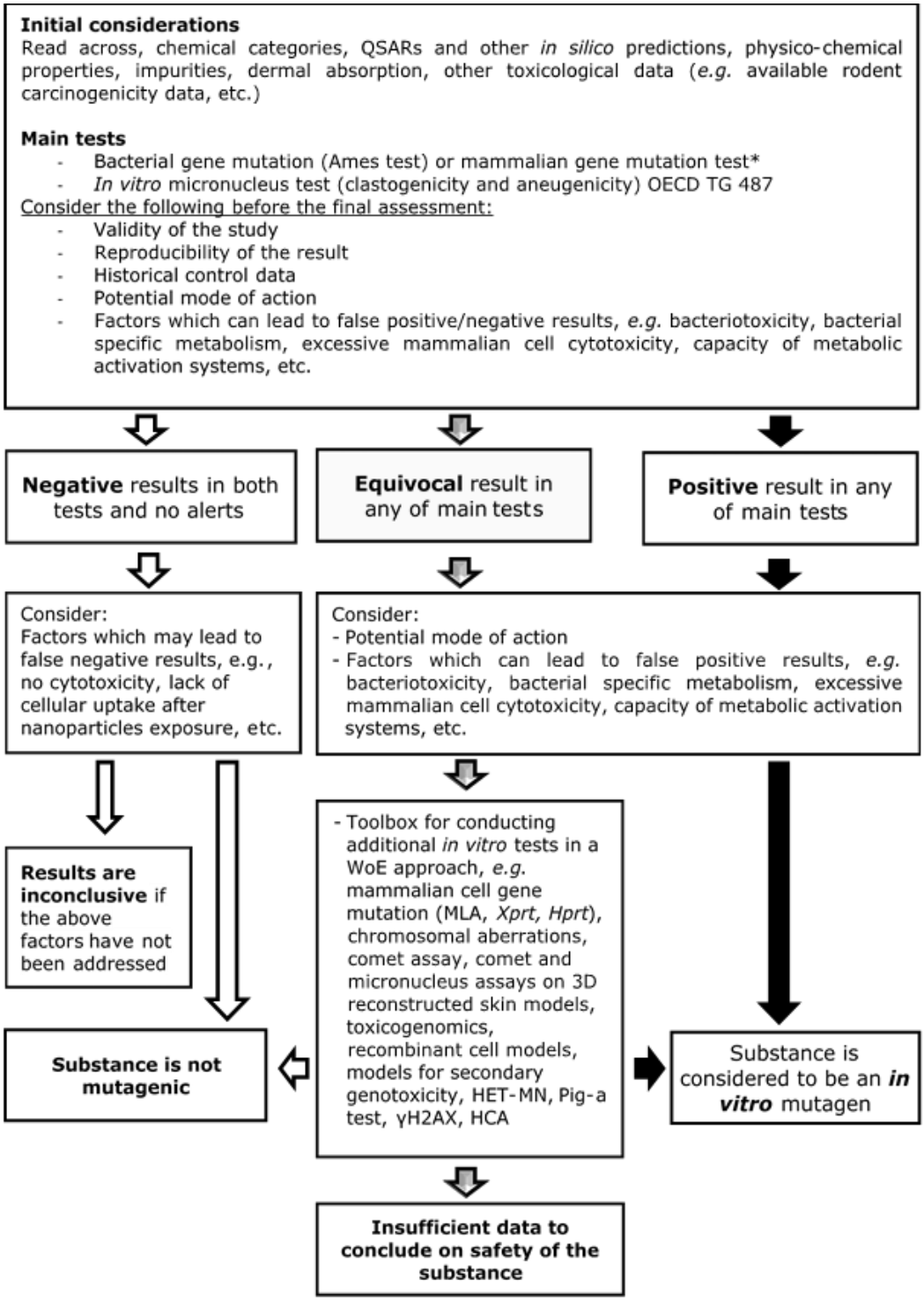
Despite the possibilities offered by the toolbox, expert judgement may be needed to be able to come to an overall conclusion.

Intensive work is being carried out on adapting current tests to high-throughput technologies (*e.g.*, micronucleus test, Comet assay, yH2AX assay, 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 GD 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 (SCCS/1532/14) to the 8th Revision of the SCCS NoG (SCCS/1501/12), in which details such as definitions, critical steps, crucial experimental conditions to be followed, etc. are described.



* Bacterial gene mutation test is preferable. If not suitable (*e.g.* in the case of antibiotics, nanomaterials), mammalian cell gene mutation assay 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; Pig-a - phosphatidylinositol glycan class A gene; γH2AX – phosphorylated form of H2A histone family member X; HCA – High Content Analysis

Figure 9. Scheme of testing strategy for genotoxicity/mutagenicity of cosmetic ingredients

B. *In vivo* methods

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).

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),

- a Mammalian Erythrocyte Pig-a Gene Mutation Assay (OECD TG 470),

- 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).

A critical factor in evaluating negative data from *in vivo* genotoxicity tests is whether the target tissue has been exposed; without evidence of adequate exposure a negative outcome may be unreliable for hazard assessment purposes. This is of particular importance where there is clear evidence of genotoxicity *in vitro.* 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 evidence of target tissue exposure should be assessed within a WoE approach. In *in vivo* MN test, evidence of target tissue exposure can be obtained in a number of different ways, as recommended by ICH S2(R1), EFSA (Hardy *et al.,* 2017), or 7th International Workshop on Genotoxicity Testing (IWGT) (Kirkland *et al*., 2019). The report of the 8th IWGT, held in Ottawa in August 2022, is not yet available.
The SCCS is aware of work being conducted in the development of new generation framework for assessment of genomic damage (Steiblen *et al.,* 2020; Luijten *et al*., 2020), however this work is at preliminary stage and no guidance can be delineated at this time.

3-4.11 Carcinogenicity

Substances are defined as carcinogenic if, after inhalation, ingestion, dermal application or injection, they induce or increase the incidence of tumours, induce 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 is *via* genotoxic effects (*i.e.* point mutations and structural chromosomal aberrations), and "non-genotoxic carcinogens", or non-DNA reactive substances that are carcinogenic due to mechanisms other than direct interactions with DNA (ECHA 2017).

A. NAMs

(i) *In silico* methods for carcinogenicity:

See under 3-4.10.2 (i): *in silico* methods for genotoxicity and carcinogenicity

(ii) *In vitro* methods

- Genotoxic carcinogens (DNA reactive)

At present validated alternative *in vitro* methods to determine the carcinogenic potential of substances are not available as OECD test Guidelines. However, there are new *in vitro* approaches that may be helpful in an overall WoE approach to indicate potential genotoxic as well as 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. If the WoE of *in vitro* mutagenicity/genotoxicity testing data indicates genotoxic activity of a substance, this 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, OECD GD 214 and OECD GD 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. One of these in particular, the TGx-DDI biomarker, was developed as a toxicogenomics signature to identify chemicals that can cause DNA damage in human cells in culture and can be used to distinguish genotoxic from non-genotoxic substances (Schmitz-Spanke, 2019; Li *et al*., 2019; Buick *et al.,* 2020, 2021).
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 to help distinguish genotoxic from non-genotoxic modes of action associated with carcinogenicity *in vitro* and may be more useful than traditional, single-endpoint tests (Wilde *et al*., 2018).

- Non-genotoxic carcinogens (DNA-non reactive)

In contrast to genotoxic carcinogens that have a mutagenic/clastogenic or aneugenic mode of action, the carcinogenic effects of NGCs may manifest from a variety of different modes of action (*e.g.* Wolf *et al.,* 2019; Cohen *et al.,* 2019), such as:

- Sustained cytotoxicity with subsequent compensatory regenerative hyperplasia;
- Receptor-mediated (*e.g.* Carboxylic Acid Reductase (CAR), Peroxisome Proliferator-activated Receptor (PPAR), Aryl Hydrocarbon Receptor (AhR);
- Induction of chronic oxidative stress;
- Induction of hormonal imbalance

In addition, many rodent tumours are considered to be not relevant to human health and have been summarised in the CLP guidance issued by ECHA (2017).

A framework for mode of action analysis for tumors seen in a rodent bioassay was published in 2001 (Sonich-Mullin *et al.,* 2001) and was later expanded upon to include evaluation of the potential human relevance of the rodent tumors (Meek *et al.,* 2003; Boobis *et al.,* 2006). The application of the human relevance framework is a critical step in determining whether rodent tumours are appropriate for use in cancer risk assessment. For those NGC that are considered to be relevant to human health, NGC are thought to have a safe exposure threshold or dose (*e.g.,* Cohen *et al*., 2019; Felter *et al.,* 2020). This highlights the importance of utilising a mode of action analysis instead of performing or relying solely on the long-term rodent carcinogenicity studies as a more direct and rational basis for human cancer risk assessment. Such analysis should be performed whenever possible, rather than simple hazard identification (Berry 2018, Goodman 2018).

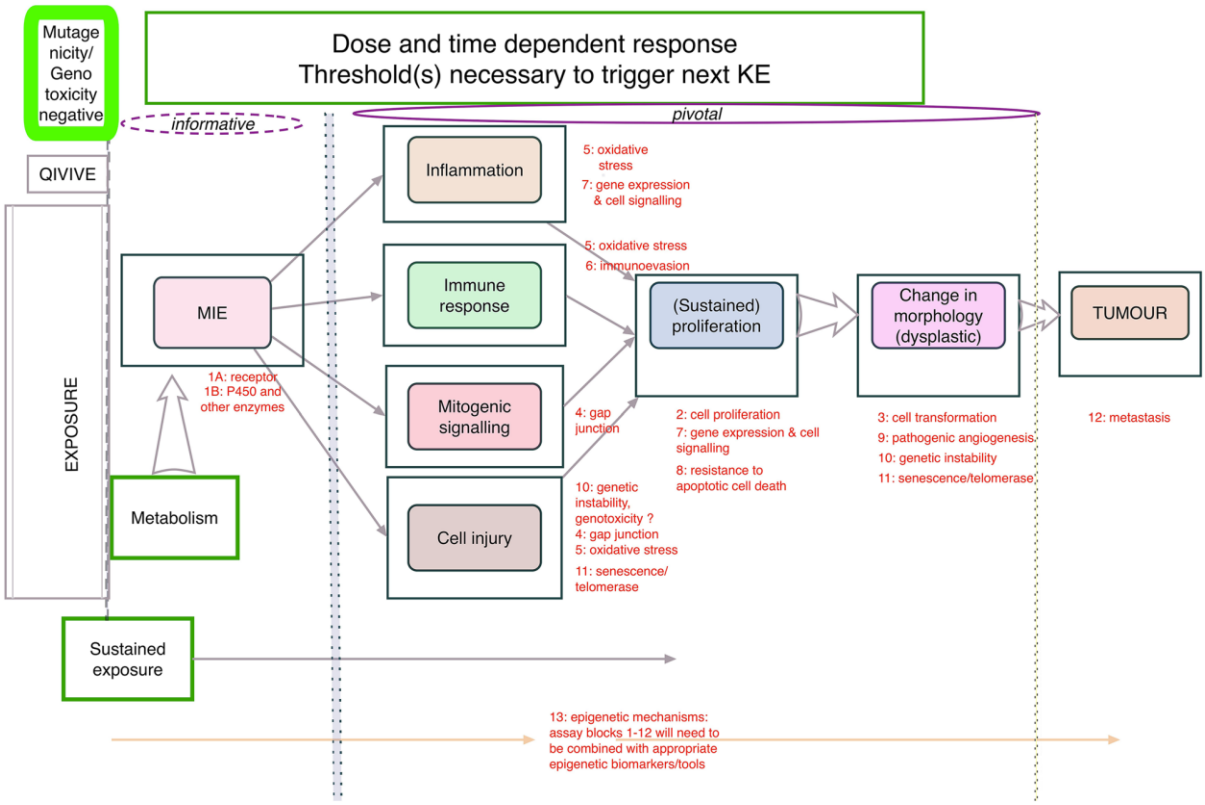
Although it has been estimated that 10-20% of recognised human carcinogens classified as Class 1 by IARC act through NGC mechanisms (Hernandez *et al.,* 2009), these fall into well-recognised categories. Of the 13 known human carcinogens considered to be non-genotoxic, five are estrogens, five are metals/organometallics, one is an immune-

suppressant, one is ethanol, and one is TCDD.
While there are ongoing efforts to develop approaches to identify NGC without a rodent bioassay, it is also recognised that these substances operate with a threshold-based mechanism such that a safe exposure limit can be identified that does not increase the risk of cancer. The overview of NGC mechanisms presented by Jacobs *et al.,* (2020) and NGC –omics markers by Oku *et al.,* (2022) indicates that assays with endpoints capturing early key event mechanisms may provide an individual contribution to the WoE approach of NGC. Dysregulation of gap junction intercellular communication (GJIC) is recognised as one of the key hallmarks for identifying non-genotoxic carcinogens (NGC). The scrape loading-dye transfer (SL-DT) technique is a simple assay for the functional evaluation of GJIC in various *in vitro* cultured mammalian cells and represents an interesting candidate assay (Sovadinová *et al.,* 2022).

(iii) Development of IATA for NGC

OECD established a programme for developing IATA for NGC (Jacobs *et al*., 2016, 2020). A general IATA of NGC is outlined in **Figure 10** (Jacobs *et al.,* 2020). Using the AOP concept, an OECD expert working group has elaborated a preliminary panel of key hallmarks of NGC and representative international standardised tests that can address IATA for NGC (Jacobs *et al*., 2020). Performing a systematic review approach combined with assay database mining, overall, more than 100 *in vitro* assays have been identified so far, within 13 cancer hallmark assay blocks that address early, mid and later key events such as receptor binding and transactivation, gene transcription, metabolism, cell proliferation, cell transformation both for early (initiation) and later (promotion) phases, with consequent increasing associations with adverse outcome. The expert group is currently evaluating these assays, including assessing their readiness for validation in the short, medium and long term.

Figure 10. A general integrated approach for the testing and assessment of non-genotoxic carcinogens (Jacobs *et al.,*2020).



(iv) Cell Transformation Assays (CTA) as a possible alternative to animal models of carcinogenicity testing

CTA can detect both genotoxic and NGC (Sasaki *et al*., 2014, Ohmori *et al.,* 2022) and are able to highlight various stages from early (initiation) to late (promotion) phases (Serra *et al*., 2019, Jacobs *et al*., 2020). They address several endpoints. They measure cell transformation, which includes early key events such as transdifferentiation, acquisition of a peculiar morphology, *etc*., reflecting stages in the multistep cancer process. CTAs thus can be used as phenotypic anchoring for mechanistic studies (Callegaro *et al*, 2017). They may provide additional information and may be used as a follow-up for confirmation of *in vitro* positive results from genotoxicity assays, typically as part of a WoE approach (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). Toxicogenomics in combination with *in vitro* CTAs allow the identification of the transcriptionally activated pathways (Mascolo *et al.*, 2018; Pillo *et al*., 2022). This integrated approach could potentially be considered as part of an IATA for non-genotoxic carcinogenesis (Corvi *et al.*, 2017).

Pre-validated CTAs are the BALB/c 3T3 CTA (EURL ECVAM, 2012), the Syrian Hamster Embryo (SHE) CTA OECD GD 214 (OECD, 2015; Corvi *et al*., 2017) and the Bhas 42 CTA OECD GD 231 (OECD, 2017; Jacobs *et al.*, 2020).

These 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. However, a recent OECD working group for developing IATA for NGC recognisedthe Bhas42 CTA as an important *in vitro* assay for the detection of NGC, as distinguished from genotoxic carcinogens (Jacobs *et al.,* 2020; Ohmori *et al.,* 2022). It has been suggested that the Bhas 42 CTA promotion test (stationary phase test) is an assay that can reproduce *in vitro* the cellular mechanisms of tumour formation and malignant transformation by NGC that cannot be detected in genotoxicity tests (Ohmori *et al.,* 2022).

In silico and *in vitro* assays to measure the key characteristics of carcinogens are summarized by Smith *et al*., 2020 and Jacobs *et al.,* 2020.

B. *In vivo* methods

An *in vivo* carcinogenicity study is only acceptable by SCCS 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.

Historically, the carcinogenic potential of a substance has been assessed using a 2-year bioassay (OECD TG 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 TG 453: combined chronic toxicity/carcinogenicity studies). It is now well recognised by the scientific and regulatory community that the use of the rodent cancer bioassay has many limitations in terms of reliability and relevance (Jacobs *et al.*, 2020).

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 TG 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 UV/VIS radiation. 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 photoclastogenicity / photomutagenicity, photo-allergy or photocarcinogenicity.

In OECD TG 432, it is indicated that if the Molar Extinction/absorption Coefficient (MEC) is less than 1000 L mol^{-1} cm^{-1} (measured in methanol), the chemical is unlikely to be photoreactive and that such chemicals may not need to be tested.

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 ranges 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.

EFSA proposed that the phototoxicity 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 optional pre-screening, the *in chemico* ROS test can be used (OECD 495).

The validated Reactive Oxygen Species (ROS) assay for photoreactivity (OECD 495) determines ROS generation from chemicals irradiated with simulated sunlight that is indicative of phototoxic potential. The applicability domain of the ROS assay is currently restricted to only those chemicals that meet the solubility criteria outlined in the protocol. Test chemicals found to be negative in the ROS assay are likely to be negative in *in vivo* test systems, whereas additional data may be required to determine if ROS photoreactive chemicals are likely to be positive *in vivo*. Some skin-lightening cosmetics may have potent reducing properties that interfere with ROS. To assess the potential for the photo-toxicity of nanomaterials, an ISO standard 20814:2019 "Nanotechnologies — Testing the photocatalytic activity of nanoparticles for NADH oxidation" is available.
As a second tier, the biological effects can be further evaluated on a reconstructed human skin model (RHE) with some barrier properties (OECD 498). A positive control should always be included. A negative result for the compound under consideration is usually accepted. Presently, no validated *in vitro* methods for the 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 being conducted on some other *in vitro* tests for photo-allergenic potential such as: photo-hCLAT, NCTC2455 assay, dendritic cell-based assay, and the photo-SH/NH2 test (Onoue *et al.,* 2017).

A Guidance document on an integrated approach on testing and assessment (IATA) for phototoxicity is expected to be approved in 2023 by the OECD Working Group of National Coordinators of the TGs programme (WNT) (Project 4.145).

B. *In vivo* methods

The data derived from *in vivo* tests should comply with the conditions mentioned in Section 1, Introduction.

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.

C. Guidance

The SCCS guidance is as follows:

- UV-VIS spectra of the compound along with the MEC, determined according to a harmonised procedure, should be provided.
- No requirement for phototoxicity testing of compounds with a MEC below 1000 $L \cdot mol^{-1}cm^{-1}$.
- There is no requirement for *in vitro* phototoxicity testing when the test material only absorbs at wavelengths lower than 313 nm and there is insufficient absorption at longer wavelengths.

As a first tier: 3T3 NRU phototoxicity test according to OECD 432 is recommended. If positive, a second tier: reconstructed human epidermis phototoxicity test according to OECD 498.

3-4.12.2 PHOTOMUTAGENICITY / PHOTOGENOTOXICITY

Photomutagenic or photogenotoxic chemicals are chemicals that absorb visible (VIS) light or UV radiation 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 (Müller and Gocke, 2013). This includes the genotoxic effects elicited by degradation products and/or radicals generated by VIS and UV wavelengths.

(i) Current status of tests available for photogenotoxicity/photomutagenicity assessment

A previous version of the Notes of Guidance (SCCNFP/0690/03) already mentioned that for the detection of photochemical clastogenicity/mutagenicity, several assays had been adapted to a combined treatment of chemicals with UV-VIS radiation (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 photomutagenicity/photogenotoxicity were 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/photomouse lymphoma assay, the photo-micronucleus test, the photochromosome 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.
In addition to the conclusions of an international workshop (Lynch *et al.,* 2011), a comprehensive review (Müller and Gocke, 2013) concluded that "photomutagenicity is not suitable for a general testing framework within cosmetic or pharmaceutical testing guidelines" and suggested a case-by-case approach.

(ii) 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, if the test is positive, no specific guidance is 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 was adopted in the EU by the Committee for Medicinal Products for Human use (CHMP) in December 2015 and issued as EMA/CHMP/ICH/752211/2012 (EMA, 2015) as well as in the USA by the 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 photogenotoxicity (Gocke *et al.,* 2000), the SCCS guidance is as follows:

- there is no requirement for a photomutagenicity test if the test material only absorbs at wavelengths lower than 313 nm and if there is insufficient absorption at longer wavelengths.
- for dermally applied compounds, in analogy to section 3-4.12.1, reconstructed skin-based phototoxicity assays could be utilised as 2nd tier assays.
- although the validity of photomutagenicity/photogenotoxicity 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 a photomutagenic/photogenotoxic hazard, then photomutagenicity 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 oxidised DNA lesions, or *in silico* methods, could be considered.

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 programmes (see Section 3-3.5.6), 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 whenthe 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 on 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 Other considerations

In safety assessment, human biomonitoring data can add important information with respect to human exposure. However, a number of limitations apply:

1. HBM is applicable to substances that are systemically taken up and where the half-life of the biomarker enables sampling and analytical determination.
2. 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.
3. HBM is not appropriate when the relevant biomarker is non-specific (*e.g.,* can be formed by different parent compounds such as hippuric acid).
4. 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.
5. 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 MoS, which is the ratio between a PoD_{sys} (usually historical NOAEL or BMD values from oral studies) and an estimate of the exposure **(10).**
Mostly, only a repeated dose toxicity study with *oral* exposure is available as a 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)}.

$$\mathbf{MoS} = \frac{\mathbf{PoD_{sys}}}{\mathbf{SED}} \qquad \mathbf{(10)}$$

3-5.1.1 THE POD VALUE

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).

3-5.1.1.1 DETERMINATION OF NOAEL

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 reproductive toxicity animal study**.

The BMD approach should preferentially be used as the dose descriptor for the PoD and the MoS calculations (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. See Section 3-1(3)(4).

3-5.1.1.2 DETERMINATION OF BMD

Although not limited to *in vivo* data, determining BMD involves first fitting a dose-response model to the data and then interpolating to find the lowest dose that causes a statistically significant response (**or alternatively**: the dose that corresponds to a low but measurable change in response over the entire dose interval). That dose is defined as the BMD. To account for uncertainty, a two-sided 90% confidence interval for the BMD interval, the BMDU (upper confidence limit of BMD), is sometimes used to calculate the BMDU/BMDL

(lower confidence limit of BMD) 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 suitable as it is does not take the full uncertainty in the BMD estimation into account (EFSA, 2017c).

With quantal data, also referred to as dose-response data, the outcomes are incidences, *e.g.* number and gender of animals with signs of toxicity. With such data, the BMD is defined as the dose associated with a specific change in the response, the 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 EFSA (EFSA, 2016).

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). Recently, EFSA published updated guidance on the use of BMD in risk assessment (EFSA, 2022).

3-5.1.1.3 CHOICE OF MODELS

BMD software (BMDS) has been developed by the US EPA (www.epa.gov/bmds) and the National Institute for Public Health and the Environment (RIVM) (the PROAST software, www.rivm.nl/proast); among other agencies, EFSA (EFSA, 2017c) has recently updated its BMD software (EFSA, 2022).

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.

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. The AIC takes the likelihood of the model fit into account but penalises models with many parameters. The SCCS considers that there are still practical considerations regarding the use of this approach when evaluating cosmetic ingredients and that its application requires a level of expert judgement and modelling expertise.

3-5.1.1.4 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.

When 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

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**[9] is also proposed (default absorption oral route: 50%; inhalation 100%; ECHA, 2012a).

3-5.1.3 MoS Analysis

The calculated MoS is compared with a reference MoS, which is comparable to the uncertainty or 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 12**). 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.

Note, however, that this way of working is only possible when historical animal data are available or new data can be derived from animal experiments that are not in contradiction with the Cosmetic Regulation (see **Appendix 1**). For application of NGRA and NAMs, other concepts will be necessary (see 3-4.1).

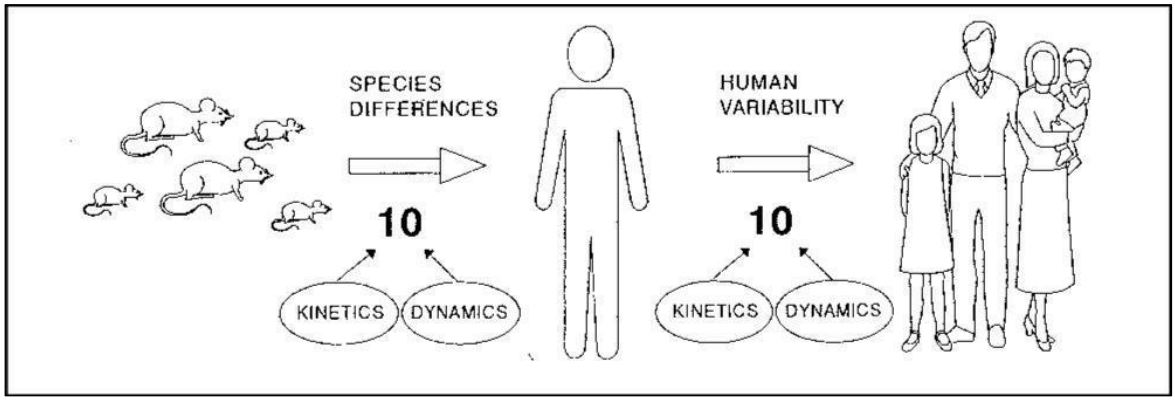


Figure 12. Schematic representation of the extrapolation from animal to man (Renwick, 1998).

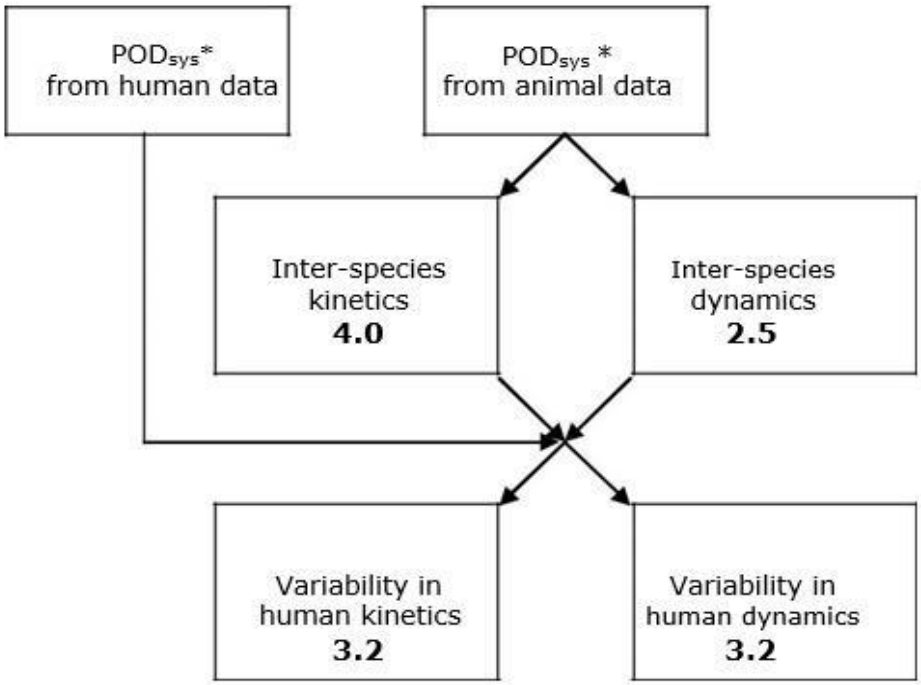
As shown in **Figure 12,** the default value of 100 consists of a factor of 10 for the extrapolation from test animals to an average human being (interspecies extrapolation) and another factor of 10 taking into account the variations within the human population (intra-species extrapolation). These factors can be further subdivided as indicated in **Figure 13.**

When considerable qualitative/quantitative toxicokinetic differences are observed between test animals and humans, as well as within human individuals, *e.g.* from relevant

[9] 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 3-3.5.2}.

toxicokinetic data for rat and/or humans (SCCS/1443/11, SCCS/1479/12), the interspecies and/or intra-species toxicokinetic default factor can be increased/decreased (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 HPT-axis disturbances in rats and humans, a change of the interspecies toxicodynamic default factor of 2.5 may be required.



* including historical NOAEL values

Figure 13. Further subdivision of the uncertainty/assessment factor, taking toxicokinetics and toxicodynamics into account (based on WHO, 1994).

Additional considerations:

- 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 once per week or once per month, for example. 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 applied once 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.
- 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 3-6.11 and 3-5.2). See also for example UV filter HAA299 (SCCS/1533/14).

- The SCCS will decide upon the relevance of MoS calculations on a case-by-case basis, taking into account the general toxicological profile of the substance under consideration, its toxicokinetic properties and its intended use**.**
- With regard to rounding and number of digits given for the MoS, this should be based on the precision of the underlying data. The biological variability of toxicity data *in vivo* generally is > 10%. The indication of more than 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 as a risk assessment tool for cosmetics and consumer products has been evaluated by the SCCS/SCHER/SCENHIR (SCCP/1171/08) as it is a pragmatic 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.
The TTC concept has been acknowledged by different organisations such as WHO IPCS, EFSA, SCCS, SCHER, Health Canada (Joint FAO/WHO Expert Committee on Food Additives, 1996; SCCP/1171/08; EFSA, 2016a & 2019a; SCCS/1564/15; and Health Canada, 2016). EFSA (EFSA, 2012 & 2019a) 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, benzidines and also 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. With respect to EDs, EFSA stated: '*In addition, once the EU-wide approach for defining and assessing low-dose effects or endocrine disrupters are finalised it will be necessary to consider any impact they may have on the use of the TTC approach*." In the ILSI monograph on TTC, Barlow disadvised the use of TTC for EDs (Barlow 2005).
So far, this approach has been used in a regulatory context for substances migrating out from food contact material, food flavourings, fragrances, genotoxic constituents in herbal preparations and for pesticide metabolites in groundwater.

It should, however, be noticed that the use of the TTC concept **for chemicals with specific data requirements for their regulatory approval** under a specific European regulation (*e.g.* Annex substances under Regulation (EC) N° 1223/2009) is currently **not acceptable as a standalone** alternative to a chemical-specific evaluation. When applied, **other lines of evidence and expert judgement** are also needed.

The TTC approach aims to screen and prioritise chemical compounds, present in **very small amounts**, for which **the chemical structure and exposure data are known,** but for which **no or limited toxicity data is available.**
An algorithm developed by Cramer (Cramer, 1978) is at the basis of the TTC concept, namely that substances, depending upon their chemical structure, were grouped into three structural classes (Class I=low, Class II=medium, Class III=high safety concern) in comparison with the toxicity data from available databases. The Carcinogen Potency Database (CPD), containing carcinogenicity data from animal studies for more than 3500 carcinogenicity experiments (Gold *et al.,* 1984) and the Munro database containing 613 chemicals based on toxicity other than carcinogenicity (Munro *et al.*, 1996) were used 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

I. Systemic toxicity

The SCCS considers the TTC approach scientifically acceptable for human health risk assessment of systemic toxic effects caused by chemicals present at **(1) very low levels** for which **(2) exposure** and **(3) chemical structure** are known.
Also, **(4) the list of chemical classes for which TTC is not applicable should be consulted** in detail in SCCP/1171/08 before applying the TTC tool.
Practical application of the TTC approach to chemicals with no genotoxicity alert is thus done by analysing their chemical structure and using Cramer classification as an indicator of systemic toxicity. As Cramer Class II is not well supported by the available database, these substances need to be treated as Class III substances. This was as such accepted (EFSA, 2016a). The application of the TTC should be done on **(5) a case-by-case basis and requires expert judgement.** The thresholds actually used are presented in **Table 10.**

Table 10: Actual thresholds for TTC application on cosmetic substances fulfilling criteria 1 to 5 as mentioned above.

Cramer class I: Substances with simple chemical structure and for which efficient modes of metabolism exist, suggesting a low order of oral toxicity.

Cramer class	**SCCP/1171/08 (Munro *et al.*, 1996)**	**Cosmos-TTC (Worth *et al.*, 2012)**	**Cosmos/Munro/ Federated DB (Yang *et al.*, 2017)**	**RIFM/Munro/Cosmos/ Federated DB (Patel *et al.*, 2020)**
Compounds lacking genotoxic alert				
I µg/kg bw/d µg/person/d	 30 1800	 42 2520	 **46*** **2760***	 49.1 2946
II*	-	-	-	-
III µg/kg bw/d µg/person/d	 1.5 90	 7.9 474	 **2.3*** **138***	 2.9 174
Potential DNA reactive mutagens and/or carcinogens				
0.0025 µg/kg bw/d and **0.15** µg/person/d ***** Values **in bold** are currently recommended by the SCCS for cosmetics-related substances.				

Cramer class II: Substances which possess structures that are less innocuous than class I substances, but do not contain structural features suggestive of toxicity like those substances in class III.

Cramer class III: Substances with chemical structures that permit no strong presumption of safety or may even suggest significant toxicity or have reactive functional groups.
From the point of view of risk assessment on the basis of non-animal data, the TTC and/or iTTC concepts will be of great value in the future. While efforts are still ongoing to further extend/ refine the TTC framework *(e.g*. for inhalation TTC and internal TTC), the SCCS considers that at present the thresholds proposed by *Yang et al.,* (2017) for Cramer class I and III are appropriate for use in relation to cosmetic-related substances.

While the use of TTC is acceptable to justify the safety of impurities and cosmetic ingredients that are added to a final product at sufficiently low concentrations, it is not acceptable on its own for the substances that are regulated under the EU Cosmetic Regulation. For this, additional supporting data from NAMs that are scientifically-accepted for the purpose, and/or other acceptable *in vivo* data on systemic toxicity, are also required in an overall weight of evidence for safety.

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 kg body weight per day and give special consideration to infants under the age of 6 months because of the potentially immature metabolism for some chemicals structures, in particular when the estimated exposure is close to tolerable exposures defined by the TTC values.

Although the TTC values are based on general toxicity data, it has been reported that datasets specific for reproductive-developmental endpoints (Laufersweiler *et al*., 2012; van Ravenzwaay *et al.*, 2017) are adequately covered. Furthermore, fragrance chemicals (238, 76 and 162 in Cramer class I, II and III, respectively) of the RIFM TTC-database were integrated in the federated dataset.

TTC thresholds are external dose-based values referring to oral systemic toxicity. For cosmetics, the main exposure route is dermal. In the proposal from Kroes *et al.* (2007) and further onwards, 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 was also an internal exposure value. This is the case under the assumption of 100% oral bioavailability, which in many cases is an overestimation.

For proper route-to-route extrapolation, the NOAELs from the Munro database need to be corrected for oral absorption. It should, however, be mentioned that quantitative information on absorption after oral administration is available in a few cases.

For botanical extracts, Kawamoto *et al.* (2019) reported that the Cramer class III TTC value of 90 µg/person/d might be adequately conservative. For potentially genotoxic substances, a TTC value of 10 µg of plant material on a dry weight basis/person per day has been proposed (Mahony *et al*., 2020). These values are not taken up in **Table 10** because plant materials are composed of mixtures.

II. Inhalation toxicity

For inhalation exposure TTC, only limited information is available (Carthew *et al.,* 2009; Escher *et al.,* 2010; Schüürmann *et al.,* 2016). Compared to the existing oral database, the pool of available repeated dose inhalation exposure studies is scarce (about 400 rodent studies and even fewer with accompanying local respiratory effects observations) (RIFM database). The development of inhalation TTC is not yet mature enough to be considered as a valid risk assessment tool.

3-5.2.3 iTTC APPROACH

For cosmetic ingredients, any risk assessment as well as the TTC approach should ideally 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. As such, several attempts have been made to arrive at an iTTC by adjusting the external NOAEL (in mg/kg bw/day) values of substances by *in silico* estimates of oral bioavailabilty (Partosch *et al*., 2015, Reilly *et al*., 2019). However, the estimates were still based on external dose and not an internal exposure metric such as plasma concentration.

Within the framework of a multi-stakeholder project, further work is currently ongoing towards the development of a set of robust iTTC values that could be utilised in human safety assessment (Rogiers *et al*., 2020). It is, however, clear that developing an iTTC database is complex and more research is required beyond current attempts where NOAELs were only adjusted for by applying *in silico* tools (Ellison *et al*., 2019; Ellison *et al.,* 2020). While work is ongoing to develop robust iTTC thresholds, an interim conservative iTTC of 1 µM plasma concentration for chemicals in consumer products has been proposed that is supported by the published experience of the pharmaceutical industry, a literature review of non-drug chemical/receptor interactions, and analysis of ToxCast™ data. This is, however, with the additional exclusion to the original TTC exclusion criteria of the estrogen and androgen receptors as targets of concern for low-dose exposures. An overview of where we are today and what is possible in the near future is given by Ellison *et al.* (2021).

3-6 SPECIAL CONSIDERATION FOR CERTAIN COSMETIC INGREDIENTS

3-6.1 Multi-constituent natural ingredients

3-6.1.1: IMPORTANT INFORMATION TO BE PROVIDED

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 (*e.g.* <5%) 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 that 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.

- Case by case, in the final safety evaluation, reference should be made to the semi-quantitative composition of the multi-constituent ingredient and the toxic potential of components should be considered.

Fragrances often have a complex composition and contact allergic reactions may occur to one of more of the ingredients. 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 Commission Regulation (EU) 2019/831 amended Annex III to Regulation (EC) No 1223/2009 (became 25 substances with the

ban of lilial on 1/3/2022). 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).

3-6.1.2 SAFETY ASSESSMENT OF COMPLEX MIXTURES

Compared to an individual cosmetic ingredient, safety assessment of botanical materials and extracts is more difficult as they are composed of a mixture of several substances - some of which may be genotoxic/carcinogenic. This is further complicated by the fact that analytical identification and characterisation of each of the chemical component of a botanical material/extract may be difficult, or even not possible. However, characterisation of a botanical material/extract is essential for safety assessment, and although no criteria have yet been agreed for botanicals, for smoke flavoring, identification and characterisation of a minimum of 50% components is required under the Commission Regulation (EC) No 627/2006.

Various strategies for assessing safety of such complex mixtures have been proposed, including the use of TTC approach. For example, Kawamoto *et al.*, (2019) reported that the Cramer class III TTC value of 90 µg/person/d might be adequately conservative for botanical extracts. For potentially genotoxic substances, a TTC value of 10 µg of plant material on a dry weight basis/person per day has been proposed (Mahony *et al.,* 2020). However, these values are not taken up in Table 10 because plant materials are composed of mixtures.

The SCCS considers that safety assessment of a botanical material/extract should involve, in the first instance, testing of the whole mixture for key toxicological endpoints - in particular genotoxicity. The TTC approach may then be considered for a botanical material/extract in accordance with the appropriate threshold for the 'negative or positive genotoxicity' substances. This should be done in a 2-pronged approach: *i.e.* for the whole material/extract, and for each of the main components that have been identified/characterised. When applying TTC:

- 1. for whole botanical material/extract, for which genotoxicity potential has been excluded but full chemical characterisation may not be available, a conservative approach would be to assume that each component belongs to Cramer class III.
- 2. for each individual component, genotoxicity testing may not be necessary if sufficient evidence can be obtained from *in silico* methods (QSAR, *read-across*) to exclude the genotoxicity potential. A more focused genotoxicity testing may then be followed for those substances for which the evidence from *in silico* methods is either inconclusive or has indicated the potential for genotoxicity.
- It needs to be re-emphasised that the use of botanical materials/extracts as cosmetic ingredients must be subjected to safety assessment, and not assumed to be 'safe' for being 'natural' or of 'plant origin'.

Avonto *et al.,* (2021) used an integrated testing strategy (ITS) for safety assessment of botanical ingredients. As a case study, they assessed the skin sensitisation potential of 30constituents of German chamomile that is used in a variety of cosmetic products (see also skin sensitisation 3-4.7)

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 of 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.

The introduction of a tiered conceptual framework for safety assessment, which starts with the collection of all available knowledge and a subsequent level in which further testing and/or data are required. The first part of the framework (collection of all available knowledge) should also take into account the concept of "history of safety use" (not equivalent to safety), and could be a practical starting point (Constable, 2007). After a comprehensive characterisation of the natural material, its origin as well as a chemical characterisation of the plant constituents under evaluation, the next step should be the comparison of the plant
material with one or more reference materials (comparators) with a known chemical/ toxicological profile. In this context, the HSU approach could help cover the safety of the unidentified part of a complex material.
Additional testing might be needed to complete the safety assessment of the botanical ingredient when robust data to support safe human use *(i.e.* chronic use as traditional medicine, dietary use) cannot be established. As many plant-derived cosmetics ingredients

have a history of human use as foods, spices and/or herbal medicines, the route of application, frequency, as well as the exposed population etc, may greatly vary. All these differences should be taken into consideration and accounted for when using historical data. Evaluated on a case-by-case basis, no standardised decision trees or ticking box approaches can be adopted for the evaluation of these complex materials.

3-6.3 Animal-derived cosmetic substances

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

Entry no. 419 in Commission Reg. (EU) 2019/831 amended Annex II of Reg. 1223/2009/EU specifies several 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*[10]."

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 a 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 TG 431, TG 439 and TG 492) (van der Valk *et al.,* 2018).

3-6.4 Sun protection substances

For sunscreen lotion, an amount of **18.0 g/day is used in the MoS calculation**. This is a standard exposure value, used in the safety evaluation by the SCCS, **but is not meant as a recommended amount to be applied by the consumer.**

The study results of Gomez-Berrada *et al.* (2017)b and (2018)b support this value (**Table 11**). In this study, consumption data were obtained from 75 clinical safety studies, conducted between 2006 and 2016, in order to assess the cutaneous tolerance and efficacy of the products. Most of the studies (57) were conducted in Mauritius, 9 studies were carried out in Spain, 8 in France and 1 in Italy. The subjects were healthy children and adults (males and females) with different skin types (normal, dry, very dry, mixed, oily, sensitive, non-sensitive, fragile, prone to atopy, with acne) and with different Fitzpatrick phototypes (I to V). For sunscreen products, people had regular sun exposure during the study.

The frequencies of use, close to real life conditions of use, were defined by the protocol as follows: at least twice a day for sunscreens, once or twice a day for moisturising cream with SPF and at least once a day for after-sun products, during 3 to 4 weeks. The amount of product used per application depended on the usage patterns specific to each participant. For young children, the cosmetic product was applied by the parents. Each tested cosmetic product was weighed at the

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

beginning and at the end of the study. The individual amount of cosmetic product used per day was calculated by dividing the total amount of product used during the study by the corresponding number of days of the study (g/day). The individual amount of cosmetic product per day was also calculated based on the exposed body surface area (mg/day/cm²). Skin surface areas were defined according to sex and age as outlined in the Exposure Factors Handbook (US EPA, 2011).

The results show that for men and women (above 15 years old), the mean amount of sunscreen applied on face and body is 8.58 g /day (equivalent to 0.46 mg/day/cm^2) and for the P95, a value of 13.03g/day (equivalent to 0.72 mg/day/cm^2) was found.

Table 11*:* Consumption and exposure assessment of suncreen products; adjusted from Gomez-Berrada *et al.,* (2017)b

	Sunscreen cream applied on face[a]						**Sunscreen cream applied on face and body[b]**				
	Amount per use		Amount per day		Exposure		Amount per use		Amount per day		Exposure
	g	mg/cm^2	g/day	mg/day/cm^2	mg/kg bw/day		g	mg/cm^2	g/day	mg/day/cm^2	mg/kg bw/day
Adult women (> 15 years old)											
Mean	1.25	2.24	2.01	3.68	34.32		3.75	0.21	8.53	0.47	139.05
SD	0.67	1.23	1.27	2.39	31.59		1.42	0.08	2.84	0.16	59.76
P50	1.11	1.96	1.75	3.14	25.28		3.54	0.19	8.44	0.47	127.96
P95	2.46	4.47	4.61	8.25	91.12		6.53	0.36	13.07	0.72	251.37
N	233	233	925	925			41	41	41	41	
Adult men and women (>15 years old)											
Mean	1.26	2.21	2.06	3.75	31.83		3.82	0.21	8.58	0.46	128.05
SD	0.66	1.23	1.25	2.39	28.32		1.52	0.08	3.13	0.17	68.01
P50	1.13	1.95	1.83	3.25	23.7		3.75	0.20	8.64	0.48	112.80
P95	2.43	4.35	4.57	8.13	83.07		6.50	0.33	13.03	0.72	256.68
N	299	299	1139	1139			62	62	62	62	

Consumption per day was expressed in g/day and in mg/day/cm²; per use in g and in mg/cm²; exposure was expressed in mg/kg bw/day.
Mean, standard deviation (SD), median (P50) and P95 values presented by age group and by sex for adults; < 10: less than 10 data; N: Number of data.
*The median value of amount per day data was applied in exposure calculation as it was not possible to adjust a distribution to the raw data. The number of amount per use data could be smaller than the number of amount per day data because in many studies (45/75), the number of use was not mentioned for each participant. In this case, the individual amount of cosmetic product per use could not be determined; [a] Cream applied on face/face and neck; [b] Cream applied on face and body.

For after-sun products, the results show that for women (above 15 years old), the amount of cream applied on face and body applied is 12.16 g/day for the mean (equivalent to 0.65 mg/day/cm^2) and 18.33 g/day for the P95 (equivalent to 0.97 mg/day/cm^2).

3-6.5 CMR Substances

Based on their inherent properties, hazardous chemicals are classified accordingly on a world-wide (Globally Harmonised System) and European level (Regulation 1272/2008). Special attention is given to substances that are *carcinogenic, germ and somatic cell*

mutagenic or toxic for reproduction for which three hazard classes exist according to these frameworks, *i.e.* Category 1A, 1B and 2.
Cat 1A: the substance is known to have the respective potential in humans;
Cat 1B: the substance is presumed to have the respective potential in humans;
Cat. 2 : the substance is suspected to have the respective potential in humans.

- In general, 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, whereby criteria are stronger for CMR 1A and 1B substances compared to CMR 2 substances.
- Evaluated by the SCCS and found safe under certain conditions, CMR 2 substances could be allowed to be used as cosmetic substances within Europe under these specific conditions. Examples for CMR2 substances include trisodium nitriloacetate (SCCS/1391/10), trimethylbenzoyldiphenylphosphine oxide (TPO) (SCCS/1528/14) polyaminopropyl biguanide (PHMB) (SCCS/1581/16), lysmeral (SCCS/1591/17), salicylic acid (SCCS/1601/18), pigmentary TiO_2 (SCCS/1617/20).

- Exceptionally, CMR 1A or 1B substances may be used in cosmetics where:

  (1) they comply with the European food safety requirements[11],
  (2) they cannot be replaced by suitable alternatives,
  (3) the application is for a particular use of the product category with a known exposure,
  (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).

This means that aggregate exposure **for CMR1** includes not only the amount of the ingredient used in all cosmetic product categories, but also the amounts coming from other sources (food, pesticides, industrial chemicals, ….) as stated in **Appendix 5**. As children are vulnerable, especially at a very young age, safety assessment based on overall exposure will be carried out, taking the different age groups into consideration.
A guidance document (**Appendix 5**) 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.
However, to provide 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 when the applicant for any reason cannot obtain the data from the owner of the data required.

3-6.6 Endocrine active substances (EAS)

3-6.6.1 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, 2013). 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 reproductive effects, metabolic disorders, cognitive deficits and cancers. 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

[11] Regulation (EC) No. 178/2002; [12] 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

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.

- The definition of Endocrine Disrupters (EDs) endorsed at the European level[12] is the same as proposed by WHO/IPCS (WHO/IPCS, 2002) and is 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".

- The joint EFSA/ECHA/JRC draft guidance (EFSA, 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 revised OECD's conceptual framework (OECD GD 150) also has a prerequisite to identify the adverse effect in an intact organism for regarding a substance as an endocrine disruptor. Thus, while 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.

- According to the Commission Delegated Regulation (2022) amending Regulation (EC) No 1272/2008 as regards hazard classes and criteria for the classification, labelling and packaging of substances and mixtures, an 'endocrine disruptor' means a substance or a mixture that alters one or more functions of the endocrine system and consequently causes adverse effects in an intact organism, its progeny, populations or subpopulations. In the CLP regulation, more definitions can be found such as 'endocrine disrupting property', 'biologically plausible link', 'endocrine disruption activity', 'endocrine disruption', and 'adverse effect'.

3-6.6.2 IDENTIFICATION OF EDS AND REGULATORY CONSEQUENCES

A number of chemicals have been identified, or are suspected, as 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).

Under REACH, EDs can be identified as Substances of Very High Concern (SVHC) alongside chemicals known to cause cancer, mutations and toxicity to reproduction. Several substances have been identified as SVHC for their endocrine disrupting properties in the Candidate List of SVHC for authorisation (https://echa.europa.eu/candidate-list-table).

Amongst other actions, the Commission launched the Fitness Check: https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1553617067256&uri=CELEX:52018DC0734 and regulated ED substances in specific areas, including chemicals (Regulation EC 1907/2006), pesticides (Regulation EC 1107/2009), biocides (Regulation EU 528/2012), water quality (2000/60/EC Water Framework Directive).
Recently, new hazard categories have been proposed to be included in the CLP Regulation (EC) No 1272/2008 (Ares, 2022). Pending that proposal, the SCCS risk assessment for a cosmetic ingredient with suspected endocrine activity will be done as follows:

When a cosmetic ingredient is suspected by the SCCS as having potential endocrine activity, safety assessment for children according to age is done, taking only the exposure for the different cosmetic categories into consideration.

[12] COM(2020) 667 final

3-6.6.3 STEPWISE APPROACH FOR COSMETICS AND THEIR INGREDIENTS

For cosmetics, the Commission adopted a review of the Cosmetics Regulation regarding substances with endocrine disrupting properties[13]. It was concluded that adequate tools are available to regulate the use of cosmetic substances that present a potential risk for human health, including when displaying ED properties. For environmental concerns, application of the REACH Regulation' is considered.
The SCCS is following this process closely and is actively engaged in the safety assessment of potential ED substances used in cosmetics.

Due to the animal testing ban under the Cosmetics Regulation, it is now out of scope to test cosmetic ingredients *in vivo* for potential endocrine disruptive effects.

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 chemico*, *in silico* models, RAx, *in vitro* assays, other mechanistic techniques such as 'omics').
Regarding "omics", it is important to consider these approaches as the first steps of identifying effects that are different for the control group and the exposed groups. For instance, metabolomics will allow comparing metabolic fingerprints of cells/tissues that were exposed to one or a cocktail of contaminants *vs.* controls. The following step consists in identifying the endogenous metabolites that are responsible for the discrimination between the different groups (if any). The next step consists in suggesting a biological hypothesis that needs to be confirmed with a targeted approach and to finally demonstrate that specific metabolic pathway(s) might be modulated by the exposure to the contaminants. Using metabolomics is very powerful and informative way to generate a hypothesis, but will not allow to conclude on either a toxic effector on an endocrine adverse effect. Metabolomics, in first instance, guide more targeted research for the identification of a mode of action (MoA).

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

The OECD 150 guidance document provides tools on how to assess the endocrine properties of a substance. The general approach taken by OECD 150 is primarily to consider the possible results that might be obtained from each ED-responsive assay and to provide guidance about how these results might be interpreted in light of data that may or may not already be available from other *in vitro* or *in vivo* assays. This should include all available data such as publications in the peer-reviewed literature as well as results from tests performed according to OECD guidelines. To provide more information for this interpretation, background data on the assays addressed, non-testing approaches and other considerations relevant to the assays are discussed. These include cross-species extrapolations, read-across and multiple MoAs. The nature, quantity and quality of the existing and new data in each of the scenarios for the ED-responsive assays should be evaluated systematically in a WoE approach. There is generally no single "right" answer. Use of other technologies (*e.g.* "omics" data) may help in understanding the link between endocrine-related mechanisms and a WoE approach. This guidance should therefore be used flexibly in light of local regulatory needs. The key questions addressed concern likely mechanisms of endocrine action and any resulting apical effects that can be attributed to such action. In **Table 12** the conceptual framework for testing and assessment of EDs as provided in OECD guidance document 150 is shown.

[13] 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.

For a new cosmetic ingredient, due to the animal testing ban, 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.

Table 12. OECD conceptual framework for testing and assessment of Eds

Level 1	Existing data and non-test information *eg* : Physical and chemical properties / QSARs
Level 2	*In vitro* assays providing data about selected endocrine mechanism(s) / pathways(s) *eg* : Estrogen receptor transactivation (OECD TG 455) / Estrogen or androgen receptor binding affinity
Level 3	*In vivo* assays providing data about selected endocrine mechanism(s) / pathway(s) *eg* : Uterotrophic assay (OECD TG 440), Fish reproductive screening assay (OECD TG 229)
Level 4	*In vivo* assays providing data on adverse effects on endocrine relevant endpoints *eg* : Repeated dose 90-day study (OECD TG 408), Daphnia reproduction test (OECD TG 211)
Level 5	*In vivo* assays providing more comprehensive data on adverse effects on endocrine relevant endpoints over more extensive parts of the life cycle of the organism *eg* : 2-generation reproduction toxicity study (OECD TG 416) / Medaka extended one-generation reproduction test (OECD TG 240)

- ***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 the Estrogen Receptor (ER), the Androgen Receptor (AR), and the 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 (EFSA, ECHA, JRC, 2018). In addition, databases are available that provide some information on endocrine properties of chemical substances[14]. These may be subject to some criticism (*e.g.,* inaccurate information, some entries not well documented, etc.). Endocrine Disruptor

[14] 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

Screening Program (EDSP) 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 a compound's endocrine activity through Danish EPA's Relative ERBA (Q)SAR, which is based on ER binding *in vitro*.
- QSAR models, including MultiCASE ERBA 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 rtnER expert system based on binding to the 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, while *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). The topic has been recently reviewed by Schneider *et al.* (2019), who highlighted that while *in silico* prediction approaches provide first stage indication of ED properties, further modeling of intermolecular interactions and cellular behavior is also essential to understand the potential effects on the endocrine system.

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

The currently available *in vitro* methods include estrogen, androgen, or steroidogenic receptor binding assays, while methods relevant to thyroid hormone are not sensitive enough to allow completely excluding 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:

i. 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).
i. Estrogen receptor transactivation (OECD TG 455),
ii. Yeast estrogen screen (ISO 19040-1,2&3)
iii. Androgen receptor transcriptional activation (OECD TG 458)
iv. Rapid androgen-disrupter activity reporter assay (draft OECD TG 251) (RADAR)
v. Steroidogenesis *in vitro* (OECD TG 456)
vi. Aromatase Assay (US EPA TG OPPT 890.1200)
vii. Thyroid disruption assays (*e.g.*, thyroperoxidase inhibition, transthyretin binding)
viii. Retinoid receptor transactivation assays
ix. Other hormone receptors assays as appropriate
x. High-throughput screens (OECD GD 211) describing Non-Guideline *In vitro* Test Methods

While the results from Levels 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, RAx, *in vitro* assays, and other techniques (such as "-omics") is integrated in a systematic manner to generate sufficient WoE to exclude the potential toxicity of a cosmetic ingredient through the endocrine-related effects. 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). Some key characteristics of EDs have also been proposed following an expert consensus statement as a basis for hazard identification (La Merill *et al.,* 2020).

3-6.6.4 SAFETY ASSESSMENT OF 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 view of the animal testing ban, the available data on these substances usually cannot comply with all five criteria as laid out under the OECD Conceptual Framework for the identification of EDs because only levels 1 and 2 are non-animal based.
If a substance is classified for its ED properties (Revision of CLP regulation) and is intended to be used in cosmetic products, some specific regulatory measures might need to be complied with in the near future. For the time being, the SCCS will treat these substances like other substances of concern for human health and therefore carry out risk 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 endocrine 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), benzophenone-3, 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).

Ingredients with potential endocrine disrupting properties used in cosmetic products are taken up in lists A and B of 28 compounds to be considered by the SCCS for safety

evaluation. 14 substances of list A are considered high priority and have been assessed by the SCCS. These are benzophenone-3 (SCCS/1625/20), kojic acid (SCCS/1637/21+corrigendum), propylparaben (SCCS/1623/20), 4-methylbenzylidene camphor (SCCS/1640/21), triclosan (SCCS/1643/22), resorcinol (SCCS/1619/20), octocrylene (SCCS/1627/21), triclocarban (SCCS/1643/22), butylated hydroxytoluene (BHT) (SCCS/1636/21), benzophenone, homosalate (SCCS/1638/21), benzyl salicylate (ongoing), genistein and daidzein (SCCS/1641/22 + corrigendum). List B is under study.

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, also be useful to support EA/ED mode of action but not their potency. For example, some ecotoxicity tests may be informative for the assessment of endocrine activity of a compound in humans or thyroid effects (*e.g.* Xenopus Eleutheroembryonic Thyroid Assay (XETA) (OECD TG 248), Amphibian Metamorphosis Assay (AMA) (OECD TG 231), Larval Amphibian Growth and Development Assay (LAGDA) (OECD TG 241) or toxicity in general (OECD TG 249).

A recent review has indicated a high degree of confidence in the conservation of the HPG-axis between fish and mammals, and the HPT-axis between amphibians and mammals (McArdle *et al.,* 2020).

An ongoing EU project ERGO (https://ergo-project.eu/) is looking into the scientific basis that could bridge the current divide between human health and the environment in terms of non-
mammalian testing for the identification of EDs (with a focus on the thyroid system) for the chemicals that affect endocrine axes across vertebrate classes.

3-6.6.5 NON -MONOTONIC DOSE RESPONSE (NMDR)

For some compounds, a so-called "non-monotonic" relationship has been observed that may bend at a particular point on the curve. Receptor saturation phenomena or a sequence of agonist/antagonist effects (Connolly & Lutz, 2004; Lagarde *et al.,* 2015) could, for example, lead to this type of relationship. This non-monotonicity has been discussed for a number of substances with ED potential (see 3-1 (3) and **Figure 2**).

3-6.7 Lifetime Cancer Risk (LCR)

In the safety assessment of carcinogenic substances, an appropriate dose descriptor, BMDL10 or T25, should be identified, whenever sufficient information is available (ECHA, 2019; EFSA, 2019b; COC, 2020). The SCCS recommends that, where possible, the BMD approach should be used for deriving a POD, as a starting point for human health risk assessment, including for carcinogenicity by a genotoxic or non-genotoxic mode of action. This view is also supported by other bodies including the EFSA and the Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment (COC). In the absence of dose-response data allowing for the application of the BMD approach, the T25 is a simplified method to estimate the carcinogenic potency of a given substance.

The T25 (expressed as mg/kg bw/d) is defined as the dose which leads to the development of tumours at a specific tissue site in 25% of the animals after correction for spontaneous incidence and within the standard lifetime of the species (Dybing *et al.*, 1997). The determination of BMDL10 (expressed as mg/kg bw/d) uses mathematical curve fitting

techniques to calculate the lower 95% confidence level at a 10% benchmark response. Both BMDL10 and T25 can be used as starting points to determine an additional LCR or to calculate a MoE, which represents the ratio between a dose descriptor and the estimated human exposure dose. Basic steps in LCR calculations based on T25 are provided in **Appendix 12.** Some countries and international organisations have considered that the LCR in the general population of less than 10^{-5} is considered tolerable (SCCS/1486/12). Under REACH, the "indicative tolerable cancer risk level" for the general population is 10^{-6} (ECHA 2012a). It should be noted that the tolerable LCR is a risk management issue and outside the scope of the mandate of the SCCS.

3-6.8 Nanomaterials

3-6.8.1 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".*

In view of the EU Chemicals Strategy for Sustainability (Ares, 2021), it is likely that the definition for a nanomaterial in the Cosmetic Regulation will be aligned with the recently published 2022/C 229/01 Commission Recommendation of 10 June 2022 on the definition of nanomaterial.

The Regulation therefore mainly covers 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.).

When dealing with the question of solubility, as provided in the current definition, it is important to note that any nano-specific risk may change (even diminish) when a nanomaterial is dissolved. But it is the time period during which the dissolution happens that determines the considerations for risk assessment based on either particle risk or soluble substance risk. Partial dissolution over a long period of time may lead to the mistaken claim that the material is 'soluble', and therefore not a nanomaterial under the scope of the current definition provided in the Cosmetic Regulation (EC) No 1223/2009.

3-6.8.2 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 market, except if they were already 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.

The SCCS was recently mandated by the Commission to provide scientific advice to facilitate the identification of any safety concerns relating to the nanomaterials intended for use in cosmetic products, so that they can be prioritised for safety assessment. The advice has recently been published (SCCS/1618/2020), which provides the key scientific

aspects of a nanomaterial that should trigger consumer safety concerns, and therefore the need for further evidence-based safety assessment.

Although there are currently no hard and fast rules for identifying the safety concerns for nanomaterials, as a general principle, each of the following attributes should add a further degree of safety concern. For example, where:

i. The nanomaterial has constituent particles that have sizes in the lower range of the nanoscale.

ii. The nanomaterial is insoluble, or only partially soluble.

iii. The chemical nature of the nanomaterial suggests the potential for a toxicological hazard.

iv. The nanomaterial has certain physical/morphological features (*e.g.* needle shape, rigid long fibres) that are associated with a higher potential for harmful effects. The nanomaterial has surface reactivity in terms of catalytic (including photocatalytic) activity, potential for radical formation, or other surface properties (*e.g.* potential allergenicity due to proteinaceous surface).

v. The nanomaterial has a different biokinetic behaviour than the conventional equivalent. For example, a surface modification/coating (*e.g.* hydrophobic coatings, encapsulation) has been applied to core nanoparticles to alter their ADME properties and as a result make them more accessible systemically, compared to the neat nanoparticles and/or their conventional chemical forms.

vi. The nanomaterial is used as vehicle to carry other substances that have not been assessed for safety as individual components, or together in the form of nano-scale entity.

vii. There is a likelihood of systemic exposure of the consumer to nanoparticles through the use of final products. The frequency of use, and/or the amounts of the relevant consumer product are relatively high.

viii. There is evidence for persistence/accumulation of nanoparticles in the body.

ix. Nanoparticles have other distinctive properties not present in conventional form of the same material, or have a new activity/function (*e.g*. a smart/functional nanomaterial).

x. The nanomaterial is so novel that it does not have a conventional comparator to allow assessment of changes in properties, behaviour or effects.

xi. The nanomaterial is used in a product that is inhalable (taken up by inhalation into respiratory tract and lung), and the particles are respirable (can reach respiratory epithelium *i.e.* alveoli).

xii. The assessment of genotoxicity is performed inadequately*, e.g. in vitro* studies are without information on stability of the test suspension, or evidence of cell exposure (internalisation).

While 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/1611/19, under revision), which is an update of a previous guidance published in 2012 (SCCS/1484/12), a Memorandum on the Relevance, Adequacy and Quality of the Data Expected in Safety Dossiers on Nanomaterials (SCCS/1524/13), 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), 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. Each of the Opinions can be consulted via the European Commission website. SCCS 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; SCCS, 2012; EC, 2012; ECHA, 2017; EFSA, 2018; EFSA, 2021a, EFSA 2021b, EC 2022).

Special features of nanomaterials:

- 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.
- 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.
- 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.
- 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.
- 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.

3-6.8.3 REQUIRED INFORMATION FOR NANOMATERIALS

The information required by the SCCS for the evaluation of nanomaterials as cosmetic ingredients is described in SCCS/1588/17 and SCCS/1611/19.

The following aspects deserve special attention:

- 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/1611/19) and EFSA (EFSA, 2011b; EFSA 2018, EFSA, 2021a, 2021b).
- 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 the solubility/degradation of the nanomaterial, the SCCS may apply a default approach and assume that 100% of the absorbed material was in nano form.
- Surface modification/surface coating may bring about profound changes in a nanomaterial in regard to certain physicochemical properties and potentially the toxic effects.
- 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.
    - Data on interaction of nanomaterial with cells (cellular uptake).

3-6.9 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 that 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).

3-6.9.1 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).

3-6.9.2 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 were drawn in the SCCS's Opinion on reaction products of oxidative hair dye ingredients formed during hair dyeing processes (SCCS/1311/10):

- Precursors and couplers with a variety of substituents such as hydroxy, amino, iminocarbonyl, hydroxyethyl, hydroxyethoxy and alkyl groups were included.
- 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 were made available for the sensitisation risk of the reaction products, this endpoint was not specifically addressed.
- The use of (Q)SAR for assessing 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. An integration of different *in silico* models provided promising results to improve the prediction of aromatic azo derivatives (Gadaleta *et al.,* 2017).
- 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 was provided under real exposure conditions.

For all the above reasons, the SCCS bases the safety assessment of oxidative hair dyes on the toxicological evaluation of the ingredients (*i.e.* precursors and couplers) and not on the reaction products.

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

3-6.10 Cosmetic ingredients for baby and children's products

3-6.10.1 AGE-RELATED DERMAL EXPOSURE

3-6.10.1.1 DEFINITIONS

"Children" are defined as 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:
Infants: 6 months -1 year
Toddlers: 1 -3 years
Children: 3-10 years
Adolescents: 10 -14 years and 14 -18 years

- 3-6.10.1.2 POTENTIAL RISK FACTORS

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).

A number of potential risk factors, however, may exist for newborns and early infants. They are reviewed in Annexes 2 and 4 of SCCS/1446/11. As dermal exposure in children is a topic of high importance for several cosmetic substances, the most important points are summarised here. An overview of potential risk factors for baby care products and their ingredients is also available in Desmedt *et al.*, 2014).

(i) The surface area/body weight ratio between children and adults: the ratio between the SSA/BW of children and adults changes from 0 to 10 years and is 2.3 at birth, 1.8 at 6 months, 1.6 at 12 months, 1.5 at 5 years, 1.3 at 10 years (Renwick, 1998).
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 and also covers variability in human kinetics (see Section 3-5.1.3). Consequently, the inter-individual variation in SSA/BW is covered by the generally accepted default value of 100 for intact skin (**Figure 10** in Section
3-5.1.3). However, for certain specific compounds the potential differences in metabolism between newborns/infants up to six months and adults could require extra consideration, but in general, the SCCS is of the opinion that there is no need for an additional UF for children **when intact skin is present** (SCCNFP/0557/02).

(ii) Toxicokinetic parameters may differ between various age groups of children and adults.

This 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 (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). These data suggest that the extent of bioactivation or metabolic toxification in children between one and ten years will in generally be different 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 and should

be considered case by case. 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 limited exposure data for newborns and early infants are available in the open literature.
Some information is available for the Netherlands at the RIVM ConsExpo Fact Sheet (2006). Data for French children have been published by Ficheux *et al.*, 2017, 2019. Exposure data for wipes used for Korean babies are available (Lee *et al.,* 2017); also for the USA, DE and UK, deterministic as well as probabilistic modelling has been carried 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). Recently, Cosmetics Europe together with Crème Global finalised a study with respect to the use of cosmetics in different age groups, including 0-3 years. The results have not yet been published. More information on how exposure data for children couldbe derived from exposure data of adults can be found in **Appendix 7**). A proposal for the different classes of cosmetic products relevant for children according to age is shown in **Appendix 7, Table A.7.2.**

(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 a somewhat higher water content in the horny layer than observed for early infants and crawlers/toddlers up to one year. Also, the variations in water content are higher. Skin pH is usually between 5-6, which is similar to the skin pH measured for adult skin. However, the nappy area is susceptible to inflammation and the buffering capacity is compromised (nappy dermatitis). This results in episodic acute skin
inflammation (mean duration 2 to 3 days) caused as well by physical, chemical and enzymatic microbial factors in the nappy environment, for example acute skin inflammation of the nappy zone occurs during changes in diet *(*breast feeding, bottle feeding, solid food) and may occur in particular between 6-12 months of age.

(v) Susceptibility against microorganisms: this is in particular the case in the nappy area and is a consequence of changes in the barrier function when the skin is damaged. Therefore, baby cosmetics should be adequately preserved (as is the case for all cosmetics) and formulated with an appropriate buffered pH.

With respect to points (i) to (iii), 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 when the skin in the nappy area is damaged** and substance-specific data clearly demonstrate that inter-individual variability results in a value higher than the default value of 10.

3-6.10.1.3 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 (diaper rash). *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 cosmetic products and the safety evaluation of the 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.* (2017).

3-6.10.2 AGE-RELATED SAFETY EVALUATION

3-6.10.2.1 GENERAL CONSIDERATIONS

Safety evaluation in the specific case of preservatives used in cosmetics for 'children' 'has been discussed for parabens (SCCS/1446/11) and phenoxyethanol (SCCS/1575/16).
The rationale of additional UFs for different age groups beyond the usual factor of 100 has been discussed in the scientific literature (*e.g.,* Renwick *et al.*, 1998 and 2000; Nielsen *et al.*, 2001; Makri *et al.*, 2004; ECHA, 2012a).

In certain cases *(e.g.* for CMRs and substances with potential endocrine activity), it is necessary to calculate the MoS of cosmetic ingredients for babies and children, *e.g.* exposure to leave-on cosmetic products designed for application on the nappy area or products intended for children with a higher sensitivity for certain endpoints (**Appendix 7, Table A.7.2**). Also, differentiation between premature babies and full-term neonates must be made since important structural and functional skin differences are present. In particular, the barrier function in premature babies is impaired (Visscher *et al.,* 2015, 2020a, 2020b). Also, pH differences play a prominent role (Fluhr and Darlenski 2018; Proksch 2018) which may be important for baby care products that are used often, such as wet wipes (Rodriguez 2020; Gustin *et al.*, 2020). Here, in the Notes of Guidance only intact skin of **full-term babies** is considered.

Seen the potential differences in metabolism between newborns/infants up to six months and older, specific exposure data to evaluate the safety of cosmetic ingredients and perform appropriate MoS calculations may be necessary. This concerns in particular oral care products, such as **toothpastes or mouthwashes**, for which the amount ingested by babies and children may be higher than for adults (see hereunder 3-6.10.2.2 and **Appendix 7, Table A.7.2**)

3-6.10.2.2 SPECIFIC EXPOSURE SCENARIOS

(i) The use of toothpaste starts with first erupted teeth and occurs with a high percentage of toothpaste ingested. Therefore, the exposure for children ages 6 years and under, as implemented for fluoride toothpastes, is generally set at a pea-size amount. The SCCNFP (SCCNFP/0653/03) defined this as 0.25 grams twice a day when assessing the safety of fluoridated oral care products for children. Furthermore, a retention factor of 40% for children 7 months - 8 years of age is recommended by the SCCS in a conservative approach (SCCS/1643/22). Above 8 years, the retention factor used is 5%. The oral

availability of the amount ingested is then considered to be 100% for babies/children as well as for adults.
(ii) The use of mouthwash potentially starts at age 6 (it is generally recommended that children under 6 should not use mouthwash). The usage volume of 21.62 g/day and retention factor of 10 % from SCCS/1628/21 is used.

(iii) Some specific exposure scenarios can be derived for children of different age categories when taken from reliable studies in which measurements under real life conditions have been done (a proposal is present in **Appendix 7, Table A.7.1**).
Default values for body weights of different age groups have been published by EFSA (EFSA 2012a), infants: 8.8 kg; toddlers: 11.9 kg; children: 23.1 kg; adolescents 10-14 yrs: 43.4 kg; adolescents 14-18 yrs: 61.3 kg).

3-6.11 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 the ingredient to be included in the finished cosmetic product
- For multi-constituent natural ingredients, with variable composition, it is essential that 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 Commission Regulation (EC) No 440/2008 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 mind 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). Also, the Applicants should keep in mind when commenting on a published preliminary Opinion by the SCCS that the comments are limited to the published text only and not considered as an opportunity to submit new data at this stage.
- When a cosmetic ingredient is present in Annex III (only allowed under strict concentrations conditions or applicability domain) and the concentration allowed is higher than when allowed as a preservative, the former concentration includes the preservative concentration *e.g.* salicylic acid.
- When aggregate exposure is calculated for the different product categories and the MoS is <100, then the industry should decide whether all concentrations are lowered in some or all product categories or one (or more) particular product category(ies) is (are) taken out.

APPENDIX 1 - INFORMATION ON REGULATION (EC) No 1223/2009 AND 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 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[15] 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.[16]

Finally, a new Commission Decision C (2015)5383[17] 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 in April 2016, for a period of 5 years until 2021, and extended until the end of 2026 due to the Covid-crisis, which postponed the launch of the call for experts/members.

[15] 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

[16] 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

[17] http://ec.europa.eu/health/scientific_committees/docs/call_2015_5383_decision_with_annexes_en.pdf

2-2 Mandate

The mission of the Scientific Committees is defined in Commission Decision C(2015)5383[18], 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.

(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, if it 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[19], 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 colourants (annex III)

[18] https://ec.europa.eu/health/sites/health/files/scientific_committees/docs/call_2015_5383_decision_with_annexes_en.pdf
[19] See Article 31 of Regulation (EC) No 1223/2009

(30) providing guidance in cooperation with relevant bodies on test methodologies which take into account specific characteristics of nanomaterials,

(32) continuously reviewing the safety of CMR substances, so that substances clarified as CMR 2 or CMR 1A or 1B can be used in cosmetics under well-restricted conditions when such use for CMR 1A and 1B has been found safe by the SCCS,

(34) taking into account the exposure of vulnerable population groups,

(35) giving opinions on the safety of use of nanomaterials in cosmetic products,

(42) consultation by the Commission as regards the applicability of validated alternative methods to the field of cosmetic products,

(49) identification of substances likely to cause allergic reactions in order that their use can be restricted and/or certain conditions can be imposed,

(61) providing assistance to the Commission as an independent risk assessment body.

The compulsory consultation of the SCCS is taken up under:

Art. 15, 2(d) and 3 for substances classified as CMR substances

Art. 16, 4 and 5 for nanomaterials

Art. 18, 2 for animal testing methodology

Art. 20, 2 for setting criteria for product claims

Art. 27, 3 for determination whether the provisional measures taken with respect to the safe clause are justified or not

Art. 31, 1 for amending Annexes II to VI for safety concerns

Art. 31, 2 for amending Annexes II to VI, VIII for technical and scientific progress

Art. 31, 3 for amending Annex I to ensure the safety of cosmetic products placed on the market.

Newly introduced modifications and improvements in the current structure and working procedures of the SCCS and the other Scientific Committee can be found in Commission Decision C(2015)5383[20] of 7 August 2015.

2-3 Rules of Procedure

The Rules of Procedure[21] 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[22] or *via* a specific call[23]). 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.

[20] https://ec.europa.eu/health/sites/health/files/scientific_committees/docs/call_2015_5383_decision_with_annexes_en.pdf
[21] https://ec.europa.eu/health/sites/health/files/scientific_committees/docs/rules_procedure_2016_en.pdf
[22] http://ec.europa.eu/health/scientific_committees/experts/database/index_en.htm
[23] http://ec.europa.eu/health/scientific_committees/open_consultation/index_en.htm

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[24].
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[25] 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[26], 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.

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[27]. 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. Recital 50 and article 18 of the Regulation,
ii. Commission Communication on the animal testing and marketing ban and on the state of play in relation to alternative methods in the field of cosmetics (COM/2013/135[28]),
iii. a factsheet of ECHA (2014a) and
iv. the 2017 ECHA report (ECHA 2017) on the use of alternatives to testing on animals.

[24] https://ec.europa.eu/health/scientific_committees/consumer_safety/requests_en
[25] https://ec.europa.eu/health/scientific_committees/consumer_safety/opinions_en#fragment0
[26] https://ec.europa.eu/health/scientific_committees/consumer_safety/opinions_en#fragment2
[27] https://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/sccnfp_opinions_97_04_en
[28] COMMUNICATION on the animal testing and marketing ban and on the state of play in relation to alternative methods in the field of cosmetics (COM(2013) 135 final).

I. Recital 50 of Regulation (EC) No 1223/2009 states the following: "*In the safety assessment of a cosmetic product 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."* The prohibitions in Article 18 of the Regulation[29] are triggered when the animal testing in question is done "*in order to meet the requirements of this* [the Cosmetics] *Regulation*". Article 18 of the Regulation (EC) No 1223/2009 creates, therefore, a relationship between the animal testing bans and the intention to meet the requirements of this Regulation. It is possible that animal testing needs to be conducted on ingredients to be used in a cosmetic product for the purpose of complying with other regulatory framework (*e.g.,* food, medicines, biocides).

II. In this respect, 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)"* . As regards the use of data from animal testing conducted to ensure compliance with non-cosmetics related legislative frameworks, two different scenarios can occur:

a. With respect to ingredients that are equally in use in other consumer and industrial products, such as in pharmaceuticals, detergents and food, animal testing may be necessary to ensure compliance with the legal frameworks applicable to these products. In this case, the Commission considers that "*the resulting animal testing data should not trigger the marketing ban and could subsequently be relied on in the cosmetics safety assessment. Reliance on such data is subject to its relevance for the cosmetics safety assessment and its compliance with data quality requirements*". However, the Commission Communication COM/2013/135 also adds that it is "*for Member States to assess and decide whether such testing for compliance with other frameworks is considered to be falling in the scope of the 2013 marketing ban"*;

b. Conversely, animal testing conducted on ingredients that have been specifically developed for cosmetic purposes and are exclusively used in cosmetic products would in the Commission's view always be assumed to be carried out in order to meet the requirements of the Regulation (EC) No 1223/2009[30], *i.e.* always be assumed to fall under the scope of the Article 18

[29] Article 18 of Regulation (EC) No 1223/2009 contains four prohibitions, two relating to the performance of animal testing (on finished cosmetic products and on ingredients of cosmetic products) and two relating to the placing on the market of cosmetic products (where the final formulation or an ingredient of a cosmetic product has been the subject of animal testing). However, an option for derogation from the animal testing ban is foreseen in Article 18, No 2, paragraph six "*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*".

[30] "*Testing carried out for cosmetics relevant endpoints on ingredients that have been specifically developed for cosmetic purposes and are exclusively used in cosmetic products would in the Commission's view always be assumed to be carried out 'in order to meet the requirements of this Directive/Regulation'*" (Commission Communication COM/2013/135, Page 8).

ban. It would not be possible, therefore, to use the results of such animal testing to prove safety of cosmetic ingredients.

III. With respect, in particular, to the interaction between REACH requirements and animal testing, ECHA published a factsheet[31] aimed at clarifying the practical meaning and implications of the Commission Communication COM/2013/135 in the context of REACH. The interface between REACH and the Regulation (EC) No 1223/2009 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[32]). For the first time, on 18 August 2020, the Board of Appeal (BoA) of ECHA took two compliance check decisions[33] on registration dossiers (for homosalate and 2-ethylhexyl salicylate, both UV filters used exclusively in cosmetics) (ECHA 2020a and 2020b) where it confirmed that, according to scientific evidence, ECHA may conclude that studies on vertebrate animals must be provided by the applicant to comply with REACH, even if the substance is used exclusively as an ingredient in cosmetics. This said, the considerations under point II above would apply, meaning that, as regards ingredients that have been specifically developed for cosmetic purposes and are exclusively used in cosmetic products, the results of a study on vertebrate animals required under REACH could not be relied upon in the cosmetic product safety report in order to demonstrate the safety for end users, as these would fall under the Article 18 ban.

However, such results will be available to the authorities for scrutiny in the cosmetic product information file under Article 11 of the Regulation (EC) No 1223/2009 and might call into question the safety of cosmetic products containing a registered substance, contradicting the cosmetic product safety report.

In this case, as mentioned by the ECHA BoA in case A-010-2018[34] "*if the safety of cosmetic products containing the substance can no longer be established, then it is possible that cosmetic products containing the substance in question as an ingredient can no longer be placed on the market"* (paragraph 112). The need to take into account the consequence of the results of that study would be justified under Article 3 of the Regulation (EC) No 1223/2009, which provides that a cosmetic product made available on the market must be safe for human health when used under normal or reasonably foreseeable conditions of use.

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

[31] https://echa.europa.eu/documents/10162/13628/reach_cosmetics_factsheet_en.pdf
[32] "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.
[33] https://echa.europa.eu/about-us/who-we-are/board-of-appeal
[34] https://echa.europa.eu/documents/10162/23010712/a-010-2018_decision_en.pdf/46612b84-29af-29ea-9192-b2506f33c8ce

V. 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)

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[35]. The Court concluded that: *''the results of animal tests, carried out outside the European Union in order to market cosmetic products in third countries, the results of which are used to prove the safety of those products for the purpose of their being placed on the EU market, must be regarded as having been carried out 'in order to meet the requirements [of that regulation]'* […]*. ''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 (SCCS/1611/19).

[35] Judgment of 21 September 2016, *European Federation for Cosmetic Ingredients*, C-592/14, ECLI:EU:C:2016:703.

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 updating the inventory in the future.
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 are 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 in 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.*
CosIng - Cosmetics - GROWTH - European Commission (europa.eu)

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:

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The SCHEER members (if applicable):

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……

……

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:
https://ec.europa.eu/transparency/regexpert/index.cfm
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

Text from the rapporteur

Or

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), which are both 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.).

Scientific Committee members

Ulrike Bernauer, Laurent Bodin, Qasim Chaudhry, Pieter Jan Coenraads, Maria Dusinska, Janine Ezendam, Eric Gaffet, Corrado Lodovico Galli, Eirini Panteri, Vera Rogiers, Christophe Rousselle, Maciej Stepnik, Tamara Vanhaecke, Susan Wijnhoven

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ISSN ISBN

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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.

SCCS - Opinions (europa.eu)

2. MANDATE FROM THE EUROPEAN COMMISSION

Background

Terms of reference

Q1
Q2
Q3

Additional information

(If appropriate)

This chapter could provide additional background information relevant to the assessment (e.g. previous Opinions or other assessments issued by other bodies/organisations).

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

3.1.1.2 Chemical names

3.1.1.3 Trade names and abbreviations

3.1.1.4 CAS / EC number

3.1.1.5 Structural formula

3.1.1.6 Empirical formula

3.1.2 Physical form

3.1.3 Molecular weight

3.1.4 Purity, composition and substance codes

3.1.5 Impurities / accompanying contaminants

3.1.6 Solubility

3.1.7 Partition coefficient (Log P_{ow})

3.1.8 Additional physical and chemical specifications

Where relevant:
- organoleptic properties (colour, odour, taste if relevant)

- melting point
- boiling point
- flash point

- vapour pressure
- density
- viscosity
- pKa
- pH
- refractive index
- UV/visible light absorption spectrum

3.1.9 Homogeneity and Stability

3.2 EXPOSURE ASSESSMENT & TOXICOKINETICS

3.2.1 Function and uses

3.2.2 Dermal / percutaneous absorption

3.2.3 Other studies on toxicokinetics

3.2.4 Calculation of SED/LED

3.3 TOXICOLOGICAL EVALUATION

3.3.1. Irritation and corrosivity

3.3.1.1 Skin irritation

3.3.1.2 Mucous membrane irritation / eye irritation

3.3.2 Skin sensitisation

3.3.3 Acute toxicity

3.3.3.1 Acute oral toxicity

3.3.3.2 Acute dermal toxicity

3.3.3.3 Acute inhalation toxicity

3.3.4 Repeated dose toxicity

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

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

3.3.4.3 Chronic (> 12 months) toxicity

3.3.5 Reproductive toxicity

3.3.5.1 Fertility and reproduction toxicity

3.3.5.2 Developmental Toxicity

3.3.6 Mutagenicity / genotoxicity

3.3.6.1 Mutagenicity / genotoxicity *in vitro*

3.3.6.2 Mutagenicity / genotoxicity *in vivo*

3.3.7 Carcinogenicity

3.3.8 Photo-induced toxicity

3.3.8.1 Phototoxicity / photo-irritation and photosensitisation

3.3.8.2 Photomutagenicity / photoclastogenicity

3.3.9 Human data

3.3.10 Special investigations

3.4 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MoS)

3.5 DISCUSSION

Physicochemical properties

Exposure & Toxicokinetics

Toxicological Evaluation

Irritation and corrosivity

Skin sensitisation

Acute toxicity

Repeated dose toxicity

Reproductive toxicity

Mutagenicity / genotoxicity

Carcinogenicity

Photo-induced toxicity

Human data

Special investigation

4. CONCLUSION

Q1
Response
Q2
Response
Q3
Response
etc

5. MINORITY OPINION

6. REFERENCES

7. GLOSSARY OF TERMS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation

8. LIST OF ABBREVIATIONS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation

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

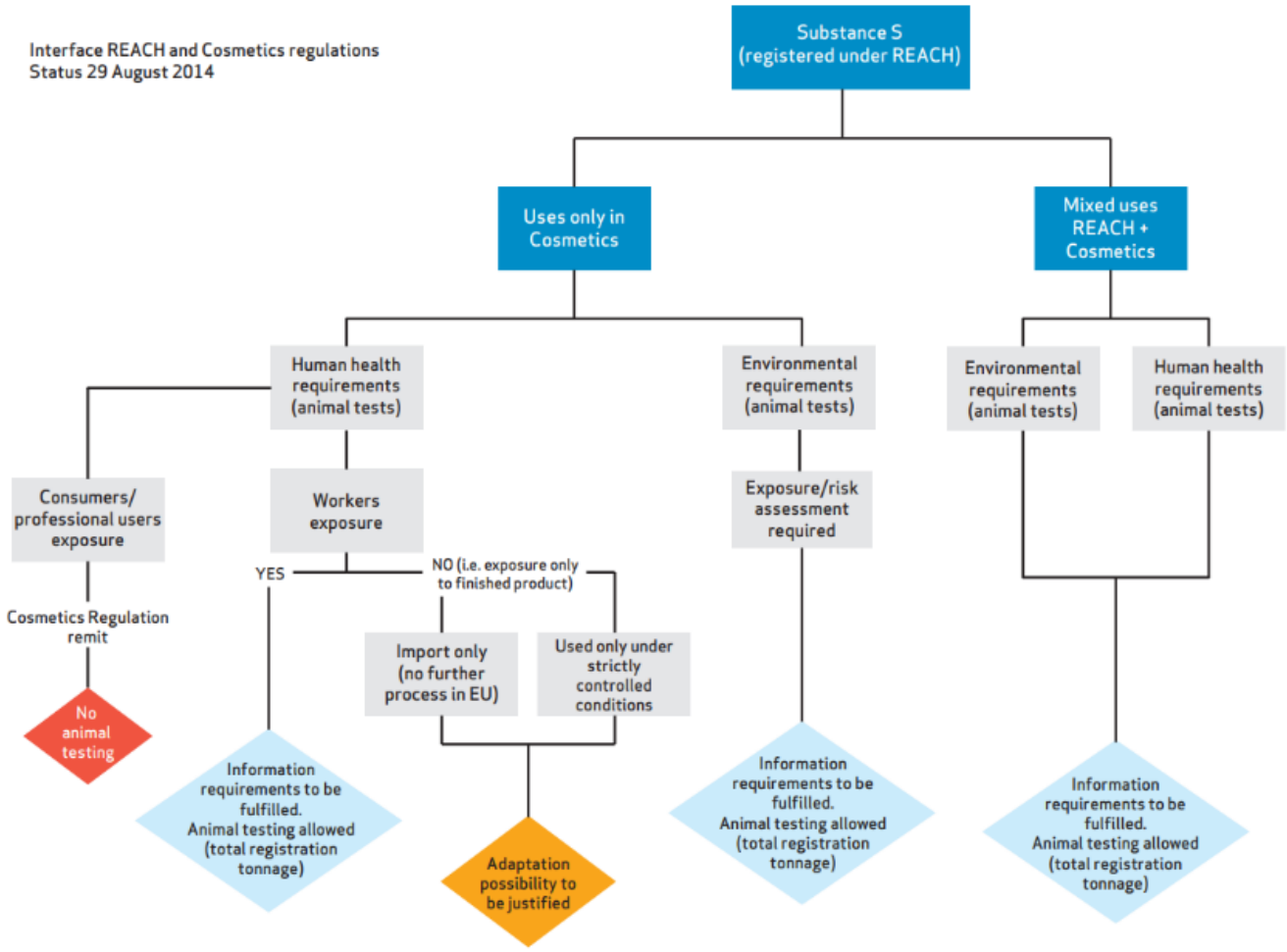

Fig A.4.1 Interface between REACH and Cosmetics regulations (ECHA, 2014a)

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[36] (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[37]. 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[38];

(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

[36] OJ L 342, 22.12.2009, p. 59.
[37] OJ L 353, 31.12.2008, p. 1.
[38] OJ L 31, 1.02.2002, p. 1.

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

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

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

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

III. Procedure

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

10. When a substance of interest for the industry is indicated in the Registry of Intentions for the purpose of its harmonised classification as CMR substance under Part 3 of Annex VI to Regulation (EC) No 1272/2008, it is for the industry to inform the Commission in due time of its intention to defend a substance under discussion 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 verify 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[39]) 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[40] and sets up the conditions under which exchange of confidential data from REACH dossiers can be ensured with ECHA.

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

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

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

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

20. The applicant should include in their submission all of the exposure information that 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.

24. 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

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

that substances used in cosmetic products may raise are considered through the application of Regulation (EC) No 1907/2006 (REACH)[41].
25. 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.

26. 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"[42].

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 light of further scientific developments.

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

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 authorised 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. This 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. 7.1,** 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. 7.1: literature overview (2015-2020) of specific cosmetic consumer exposure data and assessments

References	***Country(ies)***	***Product categories***	***Additional information***
Husoy *et al*., 2020	Norway	cosmetic products and toothpaste	Adults, both genders
Gomez-Berrada *et al.,* 2018a	France	toothpaste	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.,* 2017a	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 al*., 2016		toothpaste	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
Ficheux *et al.,*2019	France	different cosmetic products	probabilistic aggregate exposure for babies, children; 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
Manova *et al.,* 2013	Swiss,	UV-filters via skin care products	exposure
Biesterbos *et al.,* 2013	The Netherlands	Skin care products	Exposure different factors
Manová *et al.,* 2015	Swiss, Germany	UV filter ethylhexylmethoxy-cinnamate	probabilistic aggregate exposure
Tozer *et al.,* 2015	USA	Zn pyrithione in rinse-off personal cleansing products	probabilistic aggregate exposure
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

At the OECD level, some activities are running on children/children exposure. https://www.oecd.org/env/ehs/risk-assessment/childrens-health.htm

Exposure data of cosmetic products for children of different age groups is scarce.
- Recently, Cosmetics Europe with Crème global finalised a study providing exposure data for different age groups, including the age group below 3 years. Publication of this data is awaited.
- Exposure data for age groups between 4 and 14 years old is available for France (Ficheux and Roudot, 2017; Dormic *et al*., 2017a,b,c)
- Exposure data for children could also be deduced from the daily exposure data for adults taking into consideration the body surface area of adults and children, *e.g.* the exposure to preservatives used in shower gel is considered to be 190 mg/day on a surface of 17 500 cm^2 for an adult (Table 4 in 3-3.4.2.1). For a toddler of 1-3 years of age with a total body surface area of 5 600 cm^2, the daily exposure to preservatives would then result in 190 mg/d X 5 600 cm^2/17 500 cm^2 = 61 mg/day.

These calculated values have, for a number of ingredients, been compared to measured ones and it appears that they were in the same range of magnitude and for most of the product categories conservative.

In **Table A.7.2,** an example is shown of the different cosmetic product classes that are used for children of different ages.

Table A.7.2: Different cosmetic product classes to which children of different ages could be exposed

Children between 6 months and 1 year	Children between 1 and 3 years	Children between 3 and 6 years	Children between 6 and 10 years	Children between 10 and 14 years and 14 and 18 years
Shower gel Hand soap Shampoo Body lotion Face cream Hand cream	Shower gel Hand soap Shampoo Body lotion Face cream Hand cream Hair conditioner	Shower gel Hand soap Shampoo Body lotion Face cream Hand cream Hair conditioner	Shower gel Hand soap Shampoo Body lotion Face cream Hand cream Hair conditioner Mouthwash	Same as Adults
Toothpaste (RF 40%)	Toothpaste (RF 40%)	Toothpaste (RF 40%)	Toothpaste (RF 5%) Mouthwash (RF 10%)	Same as Adults

APPENDIX 8: KEY CHARACTERISTICS OF CARCINOGENS

Table A.8. Key characteristics of carcinogens (based on: Smith *et al*., 2019 and Al-Zoughool *et al.,* 2019)

Characteristic	Description
1. Is electrophilic or can be metabolically activated to electrophiles	Formation of protein adducts indicates the presence of reactive chemicals, which are sometimes also considered as indirect indicators/predictors of DNA damage (see characteristic 2, below) Requires biotransformation (metabolic activation) to produce reactive metabolites, e.g. alkylating agents, epoxide metabolites, aryl-nitrenium ion Evidence for ADME of the agent affecting its carcinogenicity
2. Is genotoxic	Direct evidence of DNA damage – this category includes nuclear and mitochondrial DNA damage (in vitro or in vivo): DNA adducts, DNA strand breaks (single- and/or double-strand breaks), DNA–protein cross-links, DNA–DNA cross-links. Indirect indicators or biomarkers of DNA damage (in vitro or in vivo). Disruption or breakages of chromosomes leading to sections of the chromosome being deleted, added, or rearranged. Reversions and forward mutations in microorganisms or mammalian cells. Mutations affecting oncogenes, tumour-suppressor genes, and other genes involved in cell cycle control.
3. Alters DNA repair or causes genomic instability	Effects on key DNA-repair mechanisms such as base-excision repair (BER) and nucleotide-excision repair (NER). Inherited abnormalities in DNA-repair function lead to enhanced cancer susceptibility.
4. Induces epigenetic alterations	Stable, long-term alterations in the transcriptional potential of a cell. These effects can be caused by factors such as altered methylation of DNA, micro-RNA expression, and changes in chromatin and histone structure.
5. Induces oxidative stress	Disturbance in the balance between the production of reactive oxygen species and antioxidant defenses within a cell.
6. Induces chronic inflammation	Chronic inflammation and/or irritation leading to oxidative DNA damage.
7. Is immunosuppressive	Measures of altered function of the immune system that may lead to increased cancer risk (e.g. HIV-related effects).
8. Modulates receptor-mediated effects	Interference with cell-signaling pathways leading to expression of carcinogenic trait/phenotype in the cell, e.g. facilitating cell invasion or induction of genes for inflammatory mediators, oncogenes Interference with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body. External agents can interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body.
9. Causes immortalization	A. Oncogenic transformation, i.e. anchorage-independent growth, loss of contact inhibition. B. Increased motility and invasiveness of cancer cell lines

	C. Cell transformation Activation of a telomerase that prevents loss of telomere length, leading to immortalization of cells.
10. Alters cell proliferation, cell death or nutrient supply	Interference with cell-signaling pathways leading to expression of carcinogenic trait/phenotype in the cell e.g. facilitating cell invasion or induction of gene promotion for inflammatory mediators, oncogenes. Induced defects in programmed cell death (apoptosis). Evasion of apoptosis is a requirement for both neoplastic transformation and sustained growth of cancer cells. Detection of alterations in cell proliferation and cell-cycle effects (e.g. DNA replication changes, cell-cycle control, ploidy), mitogenesis. Altered nutrient supply affects cell viability. Change in pro-angiogenesis factors Disruption of gap-junction intercellular communication pathways that can cause a loss of 'contact inhibition' and abnormal cell growth. The bystander effect was first identified in radiobiology and refers to the situation where non-irradiated cells exhibit effects caused by radiation as a result of chemical signals (messengers) received from nearby irradiated cells. These effects are often mediated through gap-junction transfer of chemical agents.

For representative in silico and in vitro assays to measure the key characteristics of carcinogens, see Smith et al., 2020. For key hallmarks of non-genotoxic carcinogens and representative international standardized tests, that can address these hallmarks, see Jacobs et al., 2020.

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.

APPENDIX 9: GUIDELINE ON MICROBIOLOGICAL QUALITY OF THE FINISHED COSMETIC PRODUCT

This part has been taken over from the 9th Revision of the NoG (SCCS/1564/15): https://ec.europa.eu/health/sites/health/files/scientific_committees/consumer_safety/docs/sccs_o_190.pdf

Although the NoG are concerned with the safety evaluation of ingredients, this appendix is concerned with the finished cosmetic product.
The reason for this is the fact that in other pieces of legislation, reference has been made to it as being part of the NoG.

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

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

Category 2: Other products.

Microbial contaminants usually come from two different origins: during production and filling, and during the use of the cosmetic by the consumer. From the moment the cosmetic unit is opened until the last use of the product by the consumer(s), a permanent, variable and additive microbial contamination of the cosmetic is introduced, caused by the domestic environment and contact with the skin of the consumer(s) (hands and body).
Reasons for microbial preservation of cosmetics are:
- to ensure the microbial safety of cosmetics for the consumer,
- to maintain the quality and specifications intended of the product,
- to confirm hygienic and high-quality handling.

Although only a small number of cases of microbiological contamination of cosmetics leading to microbial infections of the consumer has been reported, microbial contamination of cosmetic products may spoil them or seriously reduce the intended quality. In order to ensure the quality of the product and the safety for the consumer, it is necessary to carry out routine
microbiological analysis of each batch of the finished product coming on the market. In some justified cases *(e.g.* alcohol content > 20%), end product testing is not necessary (ISO 29621, 2010). The parameters examined, the criteria and methods used, and the results obtained per batch should be specified in properly filed reports and be taken up in the TIF.

Quantitative and qualitative limits
Quantitative and qualitative limits are based on the European Standard EN ISO 17516:2014 Cosmetics – Microbiology – Microbiological limits. The European Standard EN ISO 17516:2014 was approved by CEN on 9 August 2014 and at present is widely used

by the cosmetics industry as international standard (**Table A.9**). It is reviewed and confirmed in 2020:

Table A.9 Microbiological limits for cosmetics.
European Standard EN ISO 17516:2014 Cosmetics –Microbiology – Microbiological limits

Types of microorganism	**Products specifically intended for children under three years of age, the eye area or the mucous membranes**	**Other products**
Total Aerobic Mesophilic Microorganisms (Bacteria plus yeast and mould)	**≤ 1 x 10^2 CFU per g or ml[a]**	**≤ 1 x 10^3 CFU per g or ml[b]**
Escherichia coli	Absence in 1 g or 1 ml	Absence in 1 g or 1 ml
Pseudomonas aeruginosa	Absence in 1 g or 1 ml	Absence in 1 g or 1 ml
Staphyloccocus aureus	Absence in 1 g or 1 ml	Absence in 1 g or 1 ml
Candida albicans	Absence in 1 g or 1 ml	Absence in 1 g or 1 ml
Due to inherent variability of the plate count method, according to USP Chapter 61 or EP Chapter 2.6.12, Interpretation of results, results considered out of limit if **a > 200 CFU/g or ml,** **b > 2 000 CFU/g or ml.** NOTE When colonies of bacteria are detected on Sabouraud Dextrose agar, Sabouraud Dextrose agar containing antibiotics may be used.		

Challenge testing (based on US Pharmacopoeia 2014, European Pharmacopoeia 2014)
Note that this chapter addresses microbiological contamination, i.e. unwanted presence of microorganisms. Total germ counts and challenge test are not directly applicable for the case of probiotic cosmetic formulations to which live or viable microorganisms have been deliberately added. The efficacy of the preservation of a cosmetic product under development has to be assessed experimentally in order to ensure microbial stability and preservation during storage and use. This is done by challenge testing. The latter is mandatory for all cosmetic products that, under normal conditions of storage and use, may deteriorate or form a risk to infect the consumer.

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

It is well known today that the consistency of challenge tests relies more on the capability of the used microorganisms to contaminate a specific cosmetic product than on the taxonomic status of the microorganisms, their initial concentrations, or the conditions of incubation and media of recovery used. Microorganisms with the capability to contaminate specific cosmetics are the best candidates for use in a challenge test. The microbicidal activity of preservatives or any other compound in the finished cosmetic must be ruled out in the challenge test by dilution, filtration, addition of neutralisers or any other means.

The experimental performance of the microbial controls and the challenge tests must be carried out/supervised and validated by a microbiologist. As mentioned before, the responsible person must guarantee the efficacy of the preservation of his products experimentally by challenge testing. However, as no legal or universal challenge test method is currently available, it is up to the responsible person to decide on the details of the test to be used.

Good Manufacturing Practice (GMP)

In order to comply (mandatory but no certification required) with Good Manufacturing Practice and Microbial Quality Management, manufacturers of cosmetics haveto define and follow specific cleaning, sanitation and control procedures to keep all apparatus and materials appropriately clean and free of pathologic microorganisms. Procedures also include microbiological control of raw materials, bulk and finished products, packaging material, personnel, equipment and preparation and storage rooms. Compliance should be checked with the currently available European Committee for standardisation (CEN) standards (available through http://www.cenorm.be/cenorm/index.htm) and/or ISO standards (available through http://www.iso.org/iso/en/ISOOnline.frontpage).
According to Article 8 of Regulation (EC) No 1223/2009, good manufacturing shall be presumed where the manufacture is in accordance with the relevant harmonised standards, the references of which have been published in the Official Journal of the European Union.

APPENDIX 10: FREE ACCESS TO IN SILICO MUTAGENICITY/ GENOTOXICITY AND CARCINOGENICITY DATABASES

- The Danish QSAR database (http://qsar.food.dtu.dk/) which includes QSAR models based on structural alerts for DNA reactivity; *in vitro* Ames test in S. typhimurium, chromosome aberration in Chinese Hamster Lung (CHL) and ovary (CHO) cells; Comet assay in mouse; micronucleus test in mouse erythrocytes; sister chromatid exchange in mouse bone marrow cells; mutations in HGPRT locus in Chinese hamster ovary (CHO) cells; mutations in thymidine kinase locus in mouse lymphoma cells; and sex-linked recessive lethal test in Drosophila melanogaster.
- The OECD QSAR Toolbox (https://qsartoolbox.org/), which also incorporates the models and tools from the Danish QSAR database, provides a versatile suite of programs for chemical profiling, categorisation, and data gap filling by (Q)SAR models and read-across for various toxicological endpoints, including mutagenicity. The system also includes metabolic simulators that further enable the prediction and genotoxicity assessment of metabolites. The Toolbox also provides profilers for mutagenicity that are based on structural alerts for *in vitro* mutagenicity (Ames test), *in vivo* mutagenicity (micronucleus) chromosomal aberration and micronucleus test, and DNA and protein binding. The predictions from the profilers can provide supporting information when used in conjunction with QSAR predictions. The Toolbox also provides a few profilers that combine several structural alerts for the purpose of category formation on the basis of carcinogenicity potential of chemical substances. A notable one is the ISS profiler that combines 58 structural alerts for carcinogenicity (both genotox and non-genotox) from the Toxtree software (http://toxtree.sourceforge.net/). Around 20 of the alerts are for non-genotoxic carcinogenicity, and the remaining ones for genotoxic carcinogenicity (mutagenicity). A recent study (Aljallal, 2020) has indicated that some of the structural alerts and the profilers provided in the OECD QSAR Toolbox need further refinement, and their use
  in conjunction with QSAR models and read-across would be required to improve the accuracy of predictions.
- VEGA QSAR platform (www.vegahub.eu/) provides QSAR models for mutagenicity developed in line with the OECD principles using high quality datasets with the aim to use for regulatory purposes;
- The US-EPA's Toxicity Estimation Software Tool (T.E.S.T.) (https://www.epa.gov/chemical-research/toxicity-estimation-software-tool-test) is an Expert system that uses an ensemble of QSARs to estimate toxicity - including mutagenicity (Ames test in S. typhimurium);
- Toxtree (http://toxtree.sourceforge.net/) enables estimation of toxicity hazard by applying a decision tree approach;
- Lazar (https://lazar.in-silico.ch/predict) is an automated system of read across to calculate toxicity predictions.
- OpenTox for carcinogenicity through OpenTox platform (ToxPredict) (www.opentox.net/library/toxicity-prediction)
- OncoLogic (US EPA) (www.epa.gov/tsca-screening-tools/oncologictm-expert-system-evaluate-carcinogenic-potential-chemicals)
- Furthermore, read-across tools are available to identify similar substances and potential mechanisms of toxicity using the ToxRead and VERA software.

APPENDIX 11: INHALATION PARAMETERISATION

Table A. 11. Example for the parameterisation of a 2-Box model for sunscreens based on Rothe *et al.*, 2011 and SCCS recommendations. Product-dependent parameter values in this example that are specific for sunscreens are denoted with an asterix * (see also 3-3.5.4.1 calculation of the inhalation SED

Parameter	Parameter description	Propellant spray	Pump spray	Unit	Reference
$a_{product}$*	amount per application*	9	9	g/application	SCCS, NoG
f_{air}	air-borne fraction of spray mist	1	0.2	fraction	Bremmer *et al*, 2006b
V_1*	Box 1 (Near-field around the head)*	1000	1000	L	SCCS, Octocrylene
t_1*	duration of exposure in Box 1 (near field)*	2	2	min	SCCS, Octocrylene
r_{inh}	inhalation rate	13**	13**	L/min	US-EPA 2011
V_2*	Box 2 (Far-field, e.g. bathroom)*	10000	10000	L	SCCS, Octocrylene
t_2*	duration of exposure in Box 2 (far field)*	10	10	min	SCCS, Octocrylene
f_{resp}	respirable fraction	*experimental data*	*experimental data*	fraction	
f_{ret}	substance retention fraction in the lungs (25% exhaled)	0.75	0.75	fraction	Rothe *et al.*, 2011
f_{appl}*	frequency of application*	2	2	per day	SCCS, NoG
bw	bodyweight	60	60	kg	SCCS, NoG

* Product-dependent parameter value; **highest median among several adult age categories SCC/1627/21 Opinion on octocrylene; SCCS NoG = SCCS Notes of Guidance.

Models to estimate total and regional deposition of aerosol and/or particles

Different models are available. Examples include the Human Respiratory Tract Model (HRTM) (International Commission on Radiological Protection - ICRP, 1994, 2002), the NCRP model (National Council on Radiation Protection and Measurement) (Phalen *et al.*, 1991), the IDEAL model (Inhalation, Deposition and Exhalation of Aerosols in/from the Lung) or the MPPD model (Multiple-Path Particle Dosimetry) (Cassee *et al.*, 2002).

The ICRP human respiratory tract model is used to estimate particle penetration through the extra thoracic (ET) airways. The ICRP predictive equations for ET deposition are based on experimental measurements in humans.

The Multiple Path Particle Deposition (MPPD) model (MPPDep Version 1.11, Cassee *et al.*, 2002) allows the direct extrapolation of laboratory animal data to human exposure and is capable of estimating specific doses deposited at various sites of the respiratory tract. A dosimetric adjustment factor (DAF) is then used to convert animal exposure to human exposure, based on species-specific information on deposition, pulmonary surface area, and breathing volume. This DAF is also known as the regional deposited dose ratio (RDDR).

APPENDIX 12: LIFETIME CANCER RISK (LCR) APPROACH

The "T25 method" (Sanner *et al.*, 2001) is used as a simple method for quantitative risk assessment of carcinogens in the REACH Regulation. It should be noted that in six cases where high quality epidemiology and animal carcinogenicity studies were available, quantitative risk characterisation based on epidemiological data and data based on animal studies using the T25 method differed by factors of less than three (Sanner and Dybing, 2005).

Determination of the LCR is carried out in different steps. After having decided what animal data set to use and type of tumour to consider, the dose descriptor T25 is determined, which is described in detail (ECHA, 2012a; Dybing *et al.,* 1997).

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

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

Based on the daily lifetime SED, the LCR is calculated by linear extrapolation by use of the following formula:

$$LCR = \frac{SED}{HT25/0.25}$$

Subsequently, a statement is generated describing whether the actual risk may be higher or lower than the risk calculated for a specific scenario. The procedure and the following elements are reported and discussed in detail (Sanner *et al.*, 2001; ECHA, 2012a).

APPENDIX 13: TEMPLATES FOR PBTK ANALYSIS

The assessment of PBTK model starts by listing the general information and characteristics of PK/PBTK models that should be considered to assess the reliability of the model. These characteristics include toxicokinetic and ADME parameters (*e.g*. tissue-blood partition coefficients, metabolic constants, clearance rates) or key toxicodynamic events (*e.g.* enzyme induction, binding protein induction, cofactor depletion) (**Table A.13.1).** In a second step, evaluation of the parameters must be performed in terms of sensitivity and uncertainty analyses (**Table A.13.2**).

Table A. 13.1 - PBTK model description

PBTK model description	
Type of information	**Should contain**
Substance name	(Name, CAS number)
Authors + years of publication	
Purpose of the model	
Target population	
Route of exposure	
Dose metric selected and coherence with problem formulation	
Number, description and type of compartments	
Metabolic scheme	
Physiologicalparameter Type of parameter (e.g. tissue volumes, body weight, glomerular filtration rate, ...) Method for parameterisation	
Physico-chemical parameter Partition coefficient	
Biochemical parameter Type of parameter (e.g. metabolic rates as Vmax, Km, GEC, MET, EHR, ...) Method for parameterisation	
Model calibration	
Additional information	
Biological plausibility of the model	
Remarks	

Parameter validation and analysis
Model validation
Required information (AUC in blood, urinary excretion rates or normalised urinary content) Prediction of the selected dose metrics and ratio of dose metric prediction towards observed parameters NB: according to the IPCS guidance, the dose metric prediction must be within 2-fold of observed parameters
Additional information Description of the rational exposure scenarios (info from Risk Assessment Report might be required) Comparison of the model estimates with biomonitoring data (from literature at this stage) Simulation of potential dose dependence (e.g. testing non-linearity)
Model analysis
Sensitivity analysis performed for all parameters
Uncertainty analysis performed for the most influential parametrs

The PBTK 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 from the WHO guidance (IPCS 2010) i.e. the ratio between simulated and observed data should be 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 then be necessary to refine and update the model with further toxicokinetic (ADME) data.
Table A.13.2 - Parameter validation and analysis

Sensitivity analysis is an important component of model validation (Table A.13.2), especially for uncertain parameters with a high potential to influence the outcome of the simulation.

Uncertainty analysis evaluates the impact of the lack of precise knowledge of parameter values and model structure on dose metric simulations (IPCS 2010).

APPENDIX 14: PARAMETERS

To allow a good overview of the parameterization of exposure assessments, it is recommended to list the most important parameters and scenario considerations in tabular form at the beginning of the assessment or in the summary (**Table A.14**). For probabilistic assessments, a suitable graphical form or distribution representation needs to be used.
The list below is not exhaustive. A reference should be given for all parameter values used, as well asan explanation for their selection.

Table A. 14: Parameters of importance for exposure assessment

Parameter type	Parameter	Examples
Population	Assessed population	Exposed, total
	Age group	Adults, children 11-18, *etc.*
	Specific vulnerable group (if needed)	Pregnant women
Product-related	Concentration in product	Maximum allowed concentration
	Occurrence (use of substance in product)	Upper bound is 100%
	Retention factor	Default values given in NoG
Use related	Amount of product used Frequency of product use Body weight Inhalation rate Exposure duration Spray characteristics	
Specific parameters for inhalation	See Appendix 11	

APPENDIX 15: ABBREVIATIONS AND GLOSSARY OF TERMS

2 o 3	Two out of three
2D, 3D	Two, Three Dimensional
3R	Refinement, Reduction, Replacement
3T3 NRU PT	3T3 Neutral Red Uptake Phototoxicity Test
A	Androgen
Å	Angström
ADME	Absorption, Distribution, Metabolism, Excretion
ADRA	Amino acid Derivative Reactivity Assay
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)
AEL	Acceptable Exposure Level
AhR	Aryl hydrocarbon Receptor
AIC	Akaike Information Criterion
A.I.S.E.	International Association for Soaps, Detergents and Maintenance Products
ALI	Air Liquid Interface
Alternative methods	All those procedures which can completely replace the need for animal experiments, which can reduce the number of animals required, or which can reduce the amount of pain and stress to which the animal is subjected in order to meet the essential needs of humans and other animals (Rogiers et al., 2000; Russell et al., 1959)
AMA	Amphibian Metamorphosis Assay
AOP	Adverse Outcome Pathway
APCRA	Accelerating the PACE of Chemical Risk Assessment
AR	Androgen Receptor
Art.	Article
AhR	Aryl hydrocarbon Receptor
ATM	Alternative Test Method
ATP	Adaptation to Technical and scientific Progress
AUC	Area Under the Curve
BCOP	Bovine Corneal Opacity and Permeability
BCRP	Breast Cancer Resistance Protein
BER	Bioactivity/Exposure Ratio
BHT	Butylated HydroxyToluene
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.
BMDS	BMD Software
BMDL	BMD Lower limit refers to the corresponding lower limit of a one-sided 95% confidence interval on the BMDL
BMDU	BMD Upper limit refers to the corresponding upper limit of a one-sided 95% confidence interval on the BMD.
BMR	BenchMark Response

BoA	Board of Appeal
BP-3	Benzophenone 3
BrdU	5-Bromo-2-deoxy-Uridine
BSE	Bovine Spongiform Encephalopathy
BW	Body Weight
C	Concentration
CAR	Carboxylic Acid Reductase
CAS n°	Chemical Abstracts Service registry number
Cat.	Category
CC	Cramer Class
CEBS	Chemical Effects in Biological Systems
CEL	Consumer Exposure Level
CEN	European Committee for Standardization
CERAPP	Collaborative Estrogen Receptor Activity Prediction Project
CFU	Colony Forming Unit
CHMP	Committee for Medicinal Products for Human use
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
COC	Committee on Carcinogenicity of Chemicals in Food, Consumer Products and the Environment.
Colipa	The European Cosmetic and Perfumery Association
COM	COmmittee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment
COM	Communication from the Commission
COMET	COnsortium for MEtabonomic Toxicology
CPDB	Carcinogenic Potency DataBase
CPNP	Cosmetic Product Notification Portal
CPSR	Cosmetic Product Safety Report
CTA	Cell Transformation Assay
CVM	Collagen Vitrigel Membrane
CYP	Cytochrome P450
DA	Defined Approach
DAF	Dosimetric Adjustment Factor
DAL	Defined Approach for serious eye damage/eye irritation, Liquid
DART	Developmental and Reproductive Toxicity Database
DASS	Defined Approaches on Skin Sensitisation
DB	Data Base
DCYA	Dansylated Cysteamine
Dev.	Developmental
DG	Directorate General
DIP	Data Interpretation Procedure
DIMDI	German Institute for Medical Documentation and Information
DIT	Data Information Procedure
DPRA	Direct Peptide Reactivity Assay
E	Estrogen

EA	Substance Amount
EADB	Endocrine Activity Database
EAS	Endocrine Active Substance
EASIS	Endocrine Active Substances Information System
EATS	Estrogenic, Androgenic, Thyroid, Steroidogenic
EC	European Commission
EC3	Threshold for positive sensitization (gives a stimulation index (SI) of 3
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals 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
Ed.	Edition
E dermal	Exposure dermally
EDKB	Endocrine Disruption Knowledge Base
EDSP	Endocrine Disruption Screening Program
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
EDSP	Endocrine Disruptor Screening Program
EST	Embryonic Stem cell Test
ET	Extra Thoracic
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
fret	Retention factor
GARD	Genomic Allergen Rapid Detection
GC-MS	Gas Chromatography-Mass Spectrometry
GIVIMP	Good In Vitro Method Practices
GJIC	Gap junction intercellular communication
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice

GPMT	Guinea Pig Maximisation Test
GR	Glucocorticoid Receptor
GST	Glutathione S-Transferase
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
HCE	Human Corneal Epithelium
hCLAT	human Cell Line Activation Test
HESS	Hazard Evaluation Support System
HET-CAM	Hen's Egg Test-Chorio Allantoic Membrane
HET-MN	Hen's Egg Test for MicroNucleus induction
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
HRTM	Human Respiratory Track Model
HSDB	Hazardous Substances Data Bank
HTS	High Throughput Screening
HTS-DCYA	High Throughput Assay with Dansylated CysteAmine
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
ICRP	International Commission on Radiologic Protection
IDEAL	Inhalation,Deposition and Exhalation of Aerosols in/from the Lung
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]
IL-1?	InterLeukin-1?
INCI	International Nomenclature of Cosmetic Ingredients
INN	International Non-proprietary Name
IPCS	International Programme on Chemical Safety
IR	Infra Red
IRE	Isolated Rabbit Eye
ISSMIC	In vivo MICronucleus database
ISSSTY	In vitro mutagenesis in Salmonella TYphimurium
ISO	International Organization for Standardisation
iTTC	internal Treshold of Toxicological Concern
ITS	Integrated Testing Strategy

IUPAC	International Union of Pure and Applied Chemistry
IWGT	International Workshop on Genotoxicity Testing
JRC	Joint Research Centre
kDPRA	kinetic Direct Peptide Reactivity Assay
KE	Key Event
kNN	k-Nearest Neighbour (algorithm)
Kp	Permeation coefficient
LAGDA	Larval Amphibian Growth and Development Assay
LC50	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/m3) or as a unit volume of test article per unit volume of air (ppm, ppb)} (OECD 2009b).
LCDB	Lhasa Carcinogenicity Database
LC-MS	Liquid Chromatography-Mass Spectrometry
LCR	Lifetime Cancer Risk
LD50	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)
LED	Lowest Effective Dose, e.g. LED10
LLBO	Laser Light-Based Opacitometer
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
MAF	Mixture Assessment Factor
MDCK	Madin-Darby Canine Kidney cells
MDR	Multi Resistance Protein
MEC	Molecular Extinction Coefficient
MEGA	Multi-Endpoint Genotoxicity Assay
MIE	Molecular Initiating Event
MLA	Mouse Lymphoma Assay
MM	MicroMass
MMAD	Mass Median Aerodynamic Diameter
MN	MicroNucleus
MoA	Mode of Action
MoE	Margin of Exposure
MoS	Margin of Safety
MPPD	Multiple Path Particle Dosimeter
MR	Mitotic Recombination
mROS	micellar Reactive Oxygen Species
MRP	Multidrug Resistance-associated Protein
MS	Mass Spectrometry
MTT	3-(4,5)-diMethyl-2-Thiazolyl-2,5-dimethyl-2H-Tetrazolium bromide

MW	Molecular Weight
N	Data points
NAM	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
NCRP	National Council on Radiation Protection and measurement
NESIL	No Expected Sensitising Induction Level
NGC	Non-Genotoxic Carcinogen
NGRA	Next Generation Risk Assessment
NIH	US National Institute of Health
NIOSH	National Institute for Occupational Safety and Health
NLM	US National Library of Medicine
NLP	No Longer Polymer
NMDR	Non-Monotonic Dose Response
NMR	Nuclear Magnetic Resonance
NOAEC	No Observable Adverse Effect Concentration
NOAEL	No Observable Adverse Effect Level
NoG	Notes of Guidance
NR	Neutral Red
NRU	Neutral Red Uptake
NTP	National Toxicology Program
NURSA	NUclear Receptor Signaling Atlas
OCHEM	Online CHemical Modeling Environment
OD	Optical Density
OI	Ocular Irritection
OECD	Organisation for Economic Co-operation and Development
OPPTS	Office of Prevention, Pesticides and Toxic Substances
P50, P90	50th, 90th Percentile
PACEM	Probabilistic Aggregate Exposure
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
PDA	Photometric Diode Assay
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.)
PhEUR	European Pharmacopoeia
PHMB	PolyHexaMethylene Biguanide
PIF	Product Information File
Pig-a	Phosphatidylnositol glycan class A gene
PMS	Post-Marketing Surveillance
PoD	Point of Departure
POD sys	Point of Departure for systemic exposure
Pow	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)
PSF	Pre-Submission Form
PXR	Pregnane X Receptor
QMRF	QSAR Model Reporting Format
QRA	Quantitative Risk Assessment
QSAR	Quantitative Structure Activity Relationship
RA	Risk Assessment
RAx	Read-Across
RCPL	Reference Chemical Potency List
RDDR	Regional Deposited Dose Ratio
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
RhCE	Reconstructed human Cornea-like Epithelium test
RhE	Reconstructed human Epidermis
RhT	Reconstructed human Tissue
RIFM	Research Institute of Fragrance
RIVM	RijksInstituut voor Volksgezondheid en Milieu
rLLNA	reduced Local Lymph Node Assay
ROS	Reactive Oxygen Species
RP	Responsible Person
RSMN	Reconstructed Skin MicroNucleus assay
RTEC	Registry of Toxic Effects of Chemical substances
rtn	rainbow trout
SAF	Sensitisation Assessment Factors
SAR	Structure Activity Relationship
SARA	Skin Allergy Risk Assessment
SAS	Synthetic Amorphous Silica
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
SCHEER	Scientific Committee on Health, Environmental and Emerging Risks
SCs	Scientific Committees
SD	Standard Deviation of the mean
SED	Systemic Exposure Dose
SHE	Syrian Hamster Embryo
SI	Stimulation Index
SIN list	Substitute it Now list, made by the International Chemical Secretariat

SIT	Skin Irritation Test
SL-DT	Scrape Loading Dye Transfer
SPF	Sun Protection Factor
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).
SPSF	Standard Project Submission Form
SSA	Skin Surface Area
STE	Short Time Exposure
S	Steroidogenic
S9	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	A Serious Undesirable Effect 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)
SVHC	Substance of Very High Concern
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)
T	Thyroid
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TD	Toxicodynamic
TER	Transcutaneous Electrical Resistance
TEER	TransEpithelial Electrical Resistance
TEDX	The Endocrine Disruption Exchange
TEST	Toxicity Estimation Software Tool
TG	Test Guideline
TGR	TransGenic Rodent
TIF	Technical Information File
TK	Toxicokinetic
Tk	Thymidine Kinase
TopKat	Toxicity prediction by Komputer Assisted technology
Toxicodynamics	Cover the process of interaction of chemical substances with target sites and the subsequent reactions leading to adverse effects (ECB, 2003)
Toxicokinetics	Describe the time-dependent fate of a substance within the body and include absorption, distribution, biotransformation and/or excretion (ADME) (ECB, 2003)
TOXNET	TOXicology data NETwork
TPO	TrimethylbenzoyldiPhenylphosphine Oxide (SCCS/1528/14)
TSE	Transmissible Spongiform Encephalopathy
TTC	Threshold of Toxicological Concern
TTL	Time -To-Toxicity for liquids
TTS	Time -To-Toxicity for solids
TTT	Time -To-Toxicity
UDF	Unscheduled DNA Synthesis
UDS	Unscheduled DNA Synthesis
UF	Uncertainty Factor

UGT	Uridine diphosphate GlucuronosylTransferase
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
UPLC	Ultra High Performance Liquid Chromatography
U SENS	Myeloid U937 Skin Sensitisation Test
USA	United States of America
USP	US Pharmacopoeia
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
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
XETA	Xenopus Eleutheroembryo Thyroid Assay
XME	Xenobiotic substances Metabolising Enzyme
Xprt	Xantine-guanine phosphoribosyl transferase gene
yH2AX	Phosporylated form of H2AX histone

APPENDIX 16: LIST OF REFERENCES

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