



Scientific Committee on Consumer Safety

SCCS

MEMORANDUM on
"Alternative Test Methods
in Human Health Safety Assessment
of Cosmetic Ingredients in the European Union"



The SCCS adopted this memorandum at its 5th plenary
on 8 December 2009

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Scientific Committee members

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

Contact

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

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Prof. G. Degen
Prof. G. Eisenbrand
Prof. C.L. Galli
Prof. T. Platzek
Dr. S.C. Rastogi
Prof. V. Rogiers (chairman and rapporteur)
Prof. T. Sanner
Dr. J. van Engelen
Dr. P.M. Vinardell

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PREAMBLE

One of the mandates of the former SCC(NF)P (now SCCS) is to act as a resource of scientific expertise to the European Commission with regard to the development of 3R (refinement, reduction, replacement) alternative methods¹ and their applicability in human health safety testing of cosmetic ingredients. As such the SCCS advises the European Commission on the status of available alternative methods and their potential use in the human health risk assessment process of cosmetic ingredients and finished products.

The current EU cosmetics legislation (Council Directive 76/768/EEC²) establishes a prohibition to test finished cosmetic products and cosmetic ingredients on animals (testing ban), and a prohibition to market in the European Community, finished cosmetic products and ingredients included in cosmetic products which were tested on animals (marketing ban) (EU 1976). The testing ban on finished cosmetic products applies since 11 September 2004, whereas the testing ban on ingredients or combination of ingredients applies as of 11 March 2009, irrespective of the availability of alternative non-animal tests. The marketing ban also applies since 11 March 2009 for cosmetic products containing ingredients tested on animals. Exceptions are tests for repeated dose toxicity, reproductive toxicity and toxicokinetics. For these specific tests, the deadline of 11 March 2013 is foreseen, irrespective of the availability of alternative non-animal tests. In practical terms, this means that all studies conducted to meet the requirements of the Cosmetic Products Directive, either carried out in or outside the EU, must, after 11 March 2013, consist of validated replacement methods. Refinement (causing less suffering) and reduction (using fewer animals) alternatives are excluded as these still involve experimental animals. As the 3R-concept of Russell and Burch (1959) became a major scientific objective within the EU, several alternative methods belonging to these 3R-categories have been developed and validated. However, despite the important progress made over time (SCCNFP/0546/02, SCCNFP/0834/04, SCCP/1111/07), the number of officially validated alternative methods available for practical application in regulatory testing and risk assessment of cosmetic ingredients and finished products is still limited (Rogiers and Pauwels 2008).

The data packages for cosmetic ingredients to be included in the Annexes of the Cosmetics Directive and submitted by industry for assessment of human health safety by the SCCS, usually consist of data on identification and physico-chemical properties, acute toxicity, irritation and corrosivity (skin, eye), skin sensitisation, dermal absorption, repeated dose toxicity (28/90 days), mutagenicity/genotoxicity, developmental/reproductive toxicity and less frequently on carcinogenicity, chronic toxicity (> 12 months), toxicokinetic studies, photo-induced toxicity and information from human exposure. Until recently, most of these data were generated by animal experiments. However, analysis of the SCC(NF)P opinions between 2000 and 2009 showed that officially validated 3R-alternatives were included in the data packages whenever possible and available (Pauwels and Rogiers 2009).

Since the crucial date of 11 March 2009 has now passed, it is to be expected that in some cases, in particular for newly developed ingredients or newly performed studies, more data generated by alternative methods will be included in future data packages submitted to the SCCS. As not all new alternative methods are validated replacement methods (SCCP/1111/07), it is conceivable that safety dossiers might contain hazard information on cosmetic ingredients generated in potential conflict with the provisions of the Cosmetics Directive, (e.g. animal experiments either inside or outside the EU) or data generated for the purpose of complying with other EU (e.g. REACH) or non-EU legislations.

¹ DG XXIV/1890/98 - Mandate for SCCNFP Specific Working Group on alternative methods, for the safety evaluation of cosmetic products (XXIV/1890/98) adopted on 20 May 1998.

² As amended by Directive 2003/15/EC (EU 2003)

The responsibility of the SCCS is scientific advice in relation to human health safety. It is the applicant's responsibility to check compliance with all legal requirements. In case of doubt, the issue will be submitted to the Commission Services.

In order to actively contribute to early implementation of scientifically valid alternative methods, the SCCS closely follows up the scientific developments by academia, industry and public institutions. On a regular basis scientific meetings are organised with relevant bodies including ECVAM³, COLIPA⁴, SCHER⁵, SCENIHR⁶ and ICCG⁷ to keep knowledge on alternatives updated and to evaluate the results of validation studies and their applicability to the cosmetic sector. The advancements of EU funded Framework Programmes in the field of alternatives (Kessler 2008) are also monitored, especially the outcome of those projects that may generate potential candidate alternative methods for the full validation process.

Finally, as compound selection and the demarcation of the applicability domain of any alternative method is crucial, the SCCS is actively supporting the validation efforts of ECVAM and provided lists of candidate substances that eventually could be considered as reference compounds for the validation of *in vitro* eye irritation and skin sensitisation assays. Considering cosmetic ingredients during compound selection seems of vital importance as the Cosmetics Directive is the most stringent legislation with regard to the acceptance and use of 3R-alternatives, referring to the need for validated replacement methods.

This memorandum aims at summarizing the actual status of **officially validated** 3R-alternatives for human health safety assessment. It is acknowledged, however, that promising upcoming technologies such as predictive computational models based on a (quantitative) structure-activity relationship [(Q)SAR] approach, are under development. These models are mathematical descriptions of the biological activity of a group of chemical compounds in terms of one or more of their physicochemical properties.

A number of (Q)SAR models, developed according to quality criteria and validation principles laid down by the OECD, are available for industrial chemicals⁸ and address regulatory endpoints such as skin sensitisation, carcinogenicity, mutagenicity, developmental/reproductive toxicity and the bioconcentration factor. Likewise the so-called 'OECD QSAR ToolBox'⁹ represents a versatile suite of programs that can predict a range of endpoints for chemicals based on read-across, structural similarity, or QSAR, using a substantial set of high quality databases.

The use of (Q)SAR models as an alternative approach to testing chemical toxicity on animals has increasingly been considered by official bodies such as the ECHA (succeeding the ECB), ECVAM and the OECD. (Q)SAR models are also used in a regulatory context in the USA, where the multi-purpose tool EPISUITE¹⁰ is approved for the purpose. In Denmark, the Danish EPA has produced a comprehensive set of QSAR models for their "self classification system" of industrial chemicals. Finally, the use of (Q)SAR models is also expected to play a role in the initial stages of the implementation of REACH Regulation in the EU, in terms of filling data gaps in support of registration of chemicals.

Next to QSARs, other promising *in silico* approaches, such as physiologically based pharmacokinetic (PBPK) modelling, application of the TTC concept (see also SCCP/1171/08), read-across approaches, high-throughput screening techniques, etc., are in full development.

³ ECVAM: European Centre for the Validation of Alternative Methods

⁴ COLIPA: European Cosmetic Toiletry and Perfumery Association

⁵ SCHER: Scientific Committee on Health and Environmental Risks

⁶ SCENIHR: Scientific Committee on Emerging and Newly Identified Health Risks

⁷ ICCG: Inter-Committee Coordination Group

⁸ www.caesar-project.eu (consulted Nov 2009)

⁹ www.oecd.org/document/23/0,3343,en_2649_34379_33957015_1_1_1_1,00.html (consulted Nov 2009)

¹⁰ <http://www.epa.gov/oppt/exposure/pubs/episuite.htm> (consulted Nov 2009)

Although they are considered valuable tools for screening purposes, they currently fail to deliver the required level of knowledge for a full quantitative risk assessment. Therefore *in silico* alternative approaches are not yet a validated part of human health safety assessment of cosmetic ingredients and are not taken up in detail in the current memorandum.

1. CURRENT STATUS OF VALIDATED 3R-ALTERNATIVE METHODS FOR HUMAN HEALTH SAFETY ASSESSMENT

Validated alternatives are those methods that are in compliance with the validation process, as set up by ECVAM and its independent Advisory Committee ESAC¹¹. This means that their relevance and reliability have been established for a particular purpose, taking into account that a prediction model was present from the start of the validation process (Balls et al. 1997, Worth et al. 2001). In the meantime, the validation process has become more flexible by introducing a modular approach (Hartung et al. 2004). OECD-accepted alternative methods are also considered to be validated.

Upon compliance of a particular alternative method with all modules and after peer review by independent experts, it may be taken up in the EU legislation, more specifically in Regulation 440/2008/EC (EU 2008a).

Experience has shown that, once an alternative method has passed the validation procedure, the SCCS still may have some doubts on its applicability for the safety assessment of cosmetic ingredients. For example, it is considered important that a sufficient number of substances representative for cosmetic ingredients, included in the annexes of Directive 76/768/EEC (EU 1976), are present among the reference substances included in the validation process of the replacement alternative method under consideration. Progress could be made by incorporating relevant stakeholders (including the SCCS) at the beginning of the validation process, i.e. in the selection of the ingredients, thus providing a more efficient way of working.

1.1 Acute toxicity

Three validated alternatives for acute **oral** toxicity testing exist:

- 1) the fixed dose method (EC.B1bis, OECD 420)
- 2) the acute toxic class method (EC B.1tris, OECD 423)
- 3) the up-and-down procedure (OECD 425)

OECD Guideline 436 describes the acute toxic class method by the **inhalation** route and has recently been officially accepted (OECD 2009c).

The above alternatives are combined refinement and reduction methods, but not replacement methods.

For acute toxicity testing through the dermal route, no validated alternatives are yet available.

¹¹ ESAC: ECVAM Scientific Advisory Committee

1.2 Corrosivity and irritation of the skin

For **skin corrosion**, 5 validated replacement alternatives exist:

- 1) TER test (rat skin transcutaneous electrical resistance test) (EC B.40, OECD 430)
- 2) EpiSkin™ (EC B.40bis, OECD 431)
- 3) EpiDerm™ (EC B.40bis, OECD 431)
- 4) SkinEthic™ (EC B.40bis, OECD 431)
- 5) EST¹²-1000 (EC B.40bis, OECD 431)

Points 2) to 5) consist of commercialised reconstructed human epidermal equivalents. Although their scientific validity has been confirmed by ESAC, a recent statement of this Advisory Committee draws the attention to a newly discovered limitation of these systems with regard to the classification of volatile compounds and/or substances that display propensity to polymerisation upon contact with air (ESAC 2009a).

Nevertheless, the described *in vitro* assays form replacement alternatives for skin corrosion and although they are mainly used outside the cosmetic field, they can be useful in certain cases (e.g. acids and bases to adjust the pH of a cosmetic formulation).

For **skin irritation**, 3 validated replacement tests are available:

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

EpiSkin™ is a fully validated alternative test that passed ESAC in April 2007 (ESAC 2007). It is proposed as a stand-alone test that replaces the *in vivo* skin irritation test for the purpose of distinguishing between non-irritating and skin irritating (classified as R38 (irritating to skin) substances. The endpoint used is cell-mediated reduction of MTT [3-(4,5)-dimethyl-2-thiazolyl-2,5-dimethyl-2H-tetrazolium bromide]. The test is found useful for cosmetic ingredients by the SCCS and has recently been taken up in Part B of Commission Regulation No 440/2008 as test method B.46 (EU 2009). The concerns expressed by the SCCS with respect to interference with the colour formation by reducing substances, hair dyes and colorants (Lelièvre et al. 2007), have been taken up.

The performance of the validated EpiSkin™ test method was used for specifying ECVAM skin irritation Performance Standards (May 2007). The modified Epiderm™ SIT and the SkinEthic™ RHE test methods were subsequently validated on the basis of these Performance Standards using 20 defined Reference Chemicals (ESAC 2008) and have meanwhile been taken up in Regulation No 440/2008 (EU 2009).

In December 2008, the EU adopted Regulation No 1272/2008/EU (EU 2008b), incorporating the UN Globally Harmonized System (UN GHS) for Classification, Labelling and Packaging (CLP) of Substances and Mixtures. In agreement with the existing European system, the new EU CLP skin irritation classification system uses a single irritant category (instead of 2 in the UN GHS) and continues to use a total of 2 classification categories to distinguish irritant from non-irritant substances. However, the cut-off score shifted from an *in vivo* score of 2.0 to a value of ≥ 2.3 . The performance (specificity and sensitivity) of all three tests has been re-evaluated under the new EU CLP and was found satisfactory (ESAC 2009b).

¹² Epidermal Skin Test

1.3 Eye irritation

No fully validated alternative method for eye irritation exists. Screening methods for hazard identification (not risk assessment) to eliminate severe eye irritants are the BCOP (Bovine Cornea Opacity Permeability) (OECD 2009a) and ICE (Isolated Chicken Eye) (OECD 2009b) tests. Both tests use tissues from slaughterhouses.

The tests replace the use of experimental animals only to identify severe irritants. However, mild irritants, as often the case for cosmetic ingredients, will not be identified and not distinguished from non-irritants (<http://ecvam.jrc.it/index.htm>).

This view is supported by ICCVAM¹³ (ICCVAM 2006, ICCVAM 2007). ICCVAM recommended that the BCOP test method and the ICE test method can be used in a tiered testing strategy, as part of a weight-of-evidence approach to identify ocular corrosives and severe irritants, with specific limitations for certain chemical classes and/or physical properties. Substances that test positive in these assays can be classified as ocular corrosives or severe irritants without further testing in animals.

These tests plus two other screening tests, IRE (Isolated Rabbit Eye) and HET-CAM (Hen's Egg Test-Chorio Allantoic Membrane) are taken up in the ECB Manual of Decisions for Implementation of the 6th and 7th Amendments to Directive 67/548/EEC (EU 1967), but provide only supportive evidence for cosmetic ingredient safety assessment. ICCVAM also evaluated the IRE test method and the HET-CAM test method for this purpose. Before these two methods can be recommended for use as screening tests for the identification of ocular corrosives and severe irritants, the protocol and decision criteria for the identification of ocular corrosives and severe irritants need to be optimized and undergo further validation.

Several tests are under validation, including human reconstructed tissue models, but these are not ready yet.

Finally, a number of cytotoxicity / cell function-based assays for water soluble substances underwent retrospective validation and peer review by ESAC (ESAC 2009c):

- 1) the cytosensor microphysiometer test method (INVITTOX Protocol 102 modified) is considered suitable for:
 - discriminating ocular corrosives and severe irritants from other classes, for water-soluble molecules,
 - identifying non-irritants, as far as water-soluble surfactants and water-soluble surfactant-containing mixtures are concerned;
- 2) the fluorescein leakage test (INVITTOX Protocol 71) is suitable for discriminating ocular corrosives and severe irritants from other classes, for water-soluble chemicals (substances and mixtures);
- 3) the neutral red release (INVITTOX Protocol 54), fluorescein leakage (INVITTOX Protocols 82, 86 & 120) and red blood cell haemolysis test (INVITTOX Protocols 37 & 99) lack sufficient evidence to support a recommendation that they are ready for consideration for regulatory use.

This means that none of the above tests is suitable for determining the potency of eye irritancy.

¹³ ICCVAM: Interagency Coordinating Committee on the Validation of Alternative Methods (USA)

1.4 Skin sensitisation

The LLNA (Local Lymph Node Assay) (EC B.42, OECD Guideline 429), endorsed in 2000, is a reduction and refinement animal test. Since an allergic response does not occur after a single contact with a substance and at least a second exposure is necessary, the LLNA is considered as being a "repeated dose toxicity test".

A reduced LLNA (rLLNA) was adopted by ESAC after retrospective analysis of published data (Kimber et al. 2006). As only a negative control group and the equivalent of the high-dose group from the full LLNA are present, the rLLNA is only suitable for screening purposes to distinguish between sensitisers and non-sensitisers (<http://ecvam.jrc.it/index.htm>). The determination of the sensitising potency is not possible.

The cosmetic industry recently submitted to ECVAM a number of newly developed partial replacement *in vitro* tests for skin sensitisation. These tests have been positively assessed by ECVAM for their readiness to enter the formal validation process.

1.5 Dermal absorption

In vitro dermal absorption is described in OECD Guideline 428 (OECD 2004) and is in fact a replacement test. The guideline addresses dermal absorption from a broad point of view, wherefore the SCCNFP decided to draw up more detailed and/or stringent test requirements for cosmetics. This set of so-called 'basic criteria' was firstly published in 1999 (SCCNFP/0167/99) and has meanwhile been updated twice (SCCNFP/0750/03, SCCP/0970/06). A third update by the SCCS is underway.

The Scientific Committee considers it essential that for cosmetic ingredients not only OECD Guideline 428, but equally the additional requirements of the above-mentioned basic criteria are applied.

Absorption of a substance through the inhalation and oral route is also of importance for cosmetic ingredients (e.g. in sprays, aerosols, lipsticks and tooth paste). For both, no validated *in vitro* alternatives are available.

1.6 Repeated dose toxicity

At present, no alternative methods to replace *in vivo* repeated dose toxicity testing on experimental animals have been proposed. The SCCS is of the opinion that evaluation of the systemic risk via repeated dose toxicity testing is a key element in evaluating the safety of new and existing cosmetic ingredients. If these data are lacking in a new cosmetic ingredient submission to the SCCS, it is considered not feasible to perform risk assessment of the compound under consideration.

1.7 Mutagenicity/genotoxicity

Several *in vitro* mutagenicity/genotoxicity tests are available. An updated overview is given in the Notes of Guidance. Essentially, the SCCS recommends a battery of 3 *in vitro* assays (SCCNFP/0755/03), being:

- Bacterial Reverse Mutation Test (EC B.13/14, OECD 471)
 - *In Vitro* Mammalian Cell Gene Mutation Test (EC B.17, OECD 476)
 - *In Vitro* Micronucleus Test (OECD 487 draft);
- or
- In Vitro* Mammalian Chromosome Aberration test (EC B.10, OECD 473).

In case of clear negative results a mutagenic potential of the test compound can be excluded with sufficient certainty and negative *in vitro* results are thus considered to be highly reliable (Kirkland et al. 2007).

In contrast, positive results may be due to experimental conditions that have no relevance for the *in vivo* situation and thus do not reflect a mutagenic risk of the test compound *per se*. In order to determine whether a positive *in vitro* result has any relevance *in vivo*, follow-up testing in animals is inevitable (SCCP/1212/09). Due to the testing and marketing bans of the European legislation, it will not be possible to confirm or to exclude the mutagenic potential of candidate cosmetic ingredients with positive *in vitro* results.

There are several ongoing efforts to better define *in vitro* test conditions to avoid irrelevant positives and to improve existing tests. New *in vitro* genotoxicity assays (e.g. 3D skin models, COMET-assay) are being developed, but are not yet validated.

1.8 Carcinogenicity

For genotoxic as well as for non-genotoxic carcinogens, no validated alternative methods are available.

Cell Transformation Assays (CTA's) for the detection of chemical carcinogens, mimicking tumour formation *in vitro*, are under ECVAM validation, but the outcome is not yet available (Hayashi et al. 2008, Farmer 2002).

1.9 Reproductive Toxicity

Validated alternative methods or strategies, covering the large field of reproductive toxicity do not yet exist. Three methods have been adopted by ESAC (ESAC 2001). However, they are restricted to embryotoxicity, representing only a very limited part of reproductive toxicity. They consist of:

- the Whole Embryo Culture (WEC) test
- the MicroMass (MM) test
- the Embryotoxic Stem Cell Test (EST).

The Whole Embryo Culture (WEC) test still requires animals since pregnant animals are needed as a source of embryos.

These 3 embryotoxicity tests have not been taken up in regulatory testing.

1.10 Toxicokinetic Studies

No validated alternative methods that cover completely the field of ADME (absorption, distribution, metabolism, excretion) exist.

A number of *in vitro* models seem to be suitable to study the absorption of substances through the skin (e.g. reconstructed human epidermis) and from the gastro-intestinal tract (e.g. Caco-2 cell cultures) or provide useful information on the biotransformation of substances (e.g. isolated hepatocytes and their cultures). Although toxicokinetic data for cosmetic ingredients are only requested in certain circumstances, their relevance is high for extrapolating both *in vivo* and *in vitro* data to the human situation.

Validation is currently performed on the human HepaRG cell line and cryopreserved human and rat hepatocytes to assess their metabolic potential (cytochrome P450 activity), but results are not yet available. Moreover, hepatocyte cultures address only a small part of the ADME process.

1.11 Photo-induced Toxicity

The 3T3 Neutral Red Uptake Phototoxicity Test (3T3 NRU PT) is a validated replacement test (EC B.41, OECD 432). Besides its validation with a wide variety of chemical substances, it has also been validated using some UV-filters (Spielmann et al. 1998).

2. FURTHER CONSIDERATIONS

In recent years, progress has been made in the development and validation of alternative methods for regulatory testing of chemical substances in general, but also in the special field of cosmetic ingredients (Rogiers and Pauwels 2008). These tests, however, are primarily used for hazard identification and often do not give information on potency.

Most successes in the development of alternative methods are in local toxicity and short-term testing; they, however, often are reduction/refinement methods. The methodologies consuming the highest number of animals, however, are in long-term testing and systemic toxicity. In these fields validated alternatives and in particular validated replacement alternatives are lacking.

As experience has shown that the time needed for test development, prevalidation, validation, regulatory acceptance and use of alternative methods, is quite extensive (Eskes and Zuang 2005), the deadline of 2009 for cosmetic ingredients could not be met and the same is expected for the deadline of 2013.

Previously, serious concerns about the lack of suitable replacement methods for crucial endpoints were expressed by the SCCNFP (SCCNFP/0834/04), jointly by the CSTE and SCCNFP (CSTEE 2004), by SCCP, SCHER & SCENIHR together (ICCG 2006) and by ECVAM (ECVAM 2007).

Furthermore, nanomaterials as cosmetic ingredients (eg. UV-filters nano- ZnO and TiO₂) pose a special challenge for safety testing (e.g. as for all validated 3R-alternative tests, nanoparticle materials have never been included in the reference compounds during the validation process). This field needs special attention (SCCP/1147/07). Work is also ongoing at the SCCS and at the OECD level¹⁴.

¹⁴ There is an upcoming OECD report on the adequacy of the current guidelines to nanomaterials. It will be available at the website: http://www.oecd.org/department/0,3355,en_2649_37015404_1_1_1_1_1,00.html (consulted Sep 2009). Further information will become available in due course after the testing of 14 nanomaterials. http://www.oecd.org/document/47/0,3343,en_2649_37015404_41197295_1_1_1_1,00.html (consulted Sep 2009).

3. CONCLUSIONS

To summarize the availability and potential use of 3R-alternatives in the European cosmetic field, Table 1 displays the actual endpoints for which replacement alternatives suitable for cosmetic hazard testing, are available. In addition, it indicates which endpoints are affected by:

- The **European testing ban (2009)**, meaning that no replacement alternatives are available to provide the same level of safety insurance generated by the original *in vivo* studies that are not allowed to be performed on EU territory to meet the requirements of the Cosmetic Products Directive after 11 March 2009.
- The **European marketing ban (2009)**, meaning that no replacement alternatives are available to provide the same level of safety insurance generated by the original *in vivo* studies to which an ingredient may not be subjected after 11 March 2009 to meet the requirements of the Cosmetic Products Directive; otherwise marketing a cosmetic product containing that ingredient becomes prohibited.
- The **European marketing ban (2013)**, meaning that no replacement alternatives are available to provide the same level of safety insurance generated by the original *in vivo* studies to which an ingredient may not be subjected after 11 March 2013 to meet the requirements of the Cosmetic Products Directive; otherwise marketing a cosmetic product containing that ingredient becomes prohibited. Due to the imposed testing ban on cosmetic ingredients, *in vivo* assays falling under this category and performed between 11 March 2009 and 11 March 2013, need to be done outside the EU.

Table 1: Actual status of available replacement alternatives and impact of European cosmetic testing and marketing bans

Validated replacement alternatives available	No validated replacement alternatives available	
	→ endpoints not affected by EU testing or marketing ban	→ endpoints affected by EU testing ban (2009) EU marketing ban (2009)
<ul style="list-style-type: none"> • skin corrosivity • skin irritation • dermal absorption • mutagenicity / genotoxicity* • phototoxicity 	<ul style="list-style-type: none"> • acute toxicity • eye irritation 	<ul style="list-style-type: none"> → endpoints affected by EU testing ban (2009) EU marketing ban (2013) • repeated dose toxicity: <ul style="list-style-type: none"> - skin sensitisation - sub-acute toxicity - sub-chronic toxicity - chronic toxicity - carcinogenicity • reproductive toxicity • toxicokinetics

* Since the well-established mutagenicity / genotoxicity *in vitro* testing battery is afflicted by the frequent occurrence of false positive results, many cosmetic ingredients run the risk to wrongly be rejected due to the prohibition on *in vivo* follow-up studies (see 1.7).

Only for 5 endpoints validated replacement alternatives are available. However, for the human health risk assessment of cosmetic ingredients other endpoints are also crucial and these are not covered by meaningful and resilient replacement testing methods. In addition, the majority of the existing alternative methods is only suitable for hazard identification of cosmetic ingredients and do not give information on potency. Thus, a full human health risk assessment cannot be performed.

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