

EXECUTIVE SUMMARY

The 7th Amendment to the Cosmetics Directive

On 27 February 2003, Directive 2003/15/EC on the approximation of the laws of the EU Member States relating to cosmetics products was adopted as the 7th Amendment to the Cosmetics Directive. It introduces new provisions related to non-animal testing of cosmetic finished products and ingredients. In particular, it 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). The testing ban on finished cosmetic products will apply immediately on 11 September 2004, whereas the testing ban on ingredients or combination of ingredients will apply from 11 September 2004 as soon as alternative methods are validated by ECVAM and adopted in EU legislation, but with a maximum cut-off date of 6 years after entry into force of the Directive, i.e., 11 March 2009, irrespective of the availability of alternative non-animal tests. The marketing ban will apply from 11th September 2004 as soon as alternative methods are validated by ECVAM and adopted in EU legislation with due regard to the OECD validation process. This marketing ban will be introduced at the latest 6 years after entry into force of the Directive, i.e., 11 March 2009, for all human health effects with the exception of repeated-dose toxicity, reproductive toxicity and toxicokinetics. For these specific health effects, a deadline of 10 years after entry into force of the Directive is foreseen, i.e., 11 March 2013, irrespective of the availability of alternative non-animal tests, and which could be postponed in case of technical problems in meeting this deadline through Codecision procedure.

The Commission, after consultation of the SCCNFP and ECVAM, and with due regard to the development of validation within the OECD, shall establish timetables for the implementation of the provisions including deadlines for the phasing-out of the various animal tests. In view of establishing these timetables, the Commission decided to set up an “ad-hoc Group” between Commission services, stakeholder representatives for industry, animal welfare and consumer associations, and the OECD. The participants agreed on nominating experts on the 11 human health effects of concern in order to gain scientific expertise, and ECVAM coordinated and steered the scientific working process.

Safety data requirements for the purpose of the Cosmetics Directive

In the EU the Cosmetics Directive (76/768/EEC) imposes that: “A cosmetic product put on the market within the Community must not cause damage to human health when applied under normal or reasonably foreseeable conditions of use...”. Thus the responsibility for the product safety lies with the cosmetic manufacturer or the person placing a cosmetic product on the Community market, who must be able to demonstrate that this product is safe for the consumer. Animal testing of finished cosmetic products is not explicitly required by the Cosmetics Directive or EU Member States. However, the safety assessment of finished cosmetic products is only possible provided that an adequate toxicological data package on the ingredients is available.

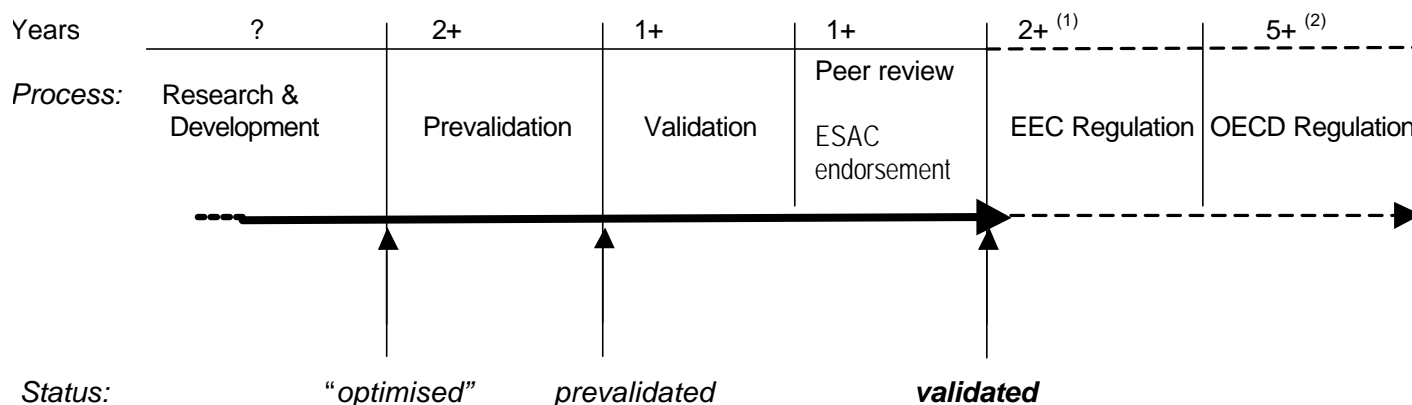
Cosmetic ingredients are substances or mixtures of substances which may be subject to testing according to the methods published in Annex V of the Dangerous Substances Directive (67/548/EEC) or in future in Annex IX of the Cosmetics Directive (Directive 2003/15/EC). The testing conducted under this legislation depends on tonnage. However, to ensure the safety of cosmetics products put on the EU market as requested by the Cosmetics Directive, all cosmetic ingredients, regardless of tonnage must have appropriate data to permit an adequate safety assessment. Since the Cosmetics Directive does not specify a fixed data set or methods that are needed when assessing the safety of cosmetic ingredients, guidance is given by the SCCNFP with regard to the areas of potential toxicity that need to be addressed. Finally, the safety assessment of a cosmetic product requires taking into account the general profile of its ingredients, i.e. not only their hazard (which is the intrinsic potential of a substance to cause damage to human health and allows for classification and labelling of the substance), but also, and most importantly, the risk to human health (which is the probability of a substance to cause damage under relevant use conditions; this can be evaluated in light of dose-response studies and assessment of the actual exposure).

Experts’ review and time estimation for phasing-out animal tests

Eleven subgroups were formed according to the 11 human health effects of concern. These subgroups comprised experts representing the different stakeholders, i.e. industry, academia, animal welfare associations and governmental bodies. In order to estimate the time necessary to achieve full replacement of animal testing in the field of cosmetics, the participating experts were requested in a first step, to provide an inventory of the most valuable and/or advanced alternative methods currently known to be available in the respective toxicological areas. Based on this inventory, they were requested in a second step to:

- Estimate the time necessary to achieve ESAC endorsement of the identified methods assuming that optimal conditions are met
- Identify the gaps left by the *in vitro* methods compared to the animal test
- Give recommendations for achieving full animal replacement
- Estimate the time necessary, including regulatory acceptance, to achieve full replacement of animal test assuming that all necessary conditions are optimally met (e.g., funding, human resources, etc).

The experts based their judgement on the time necessary to obtain validation and regulatory acceptance of alternative methods using the following time frame (the years given represent durations under optimal conditions, while the plusses indicate that some unforeseeable delays often occur):



The conclusions reached by the different expert panels are summarized hereafter. It is important to note that the evaluation of the time necessary to achieve full replacement of animal tests often represents a compromise reached by the broad representation of experts rather than full agreement.

1. Acute toxicity

Good progress has been made on alternative methods for predicting basal cytotoxicity, which is one component of the acute toxicity potential. Several studies such as the MEIC study have shown that cell culture, and in particular human cells, could be used for the measurement of basal cytotoxicity. Amongst those, the experts identified 8 well-advanced *in vitro* tests capable of predicting basal cytotoxicity. For some of these validation and ESAC endorsement could be achieved in 2 to 4 years. However, for replacing the whole animal test, other

⁽¹⁾ For details see Appendix 3

⁽²⁾ For details see Appendix 4

parameters, such as metabolism, toxicokinetics and target organ toxicity to potentially sensitive target organs, need to be taken into account. Efforts to address these parameters are still at a research level. Once methods are developed and validated, there will still be the need to integrate the individual test components into a validated test battery combined with a prediction model for data extrapolation to define the risk relevant to humans. A proposal for an integrated project (A-Cute-Tox) has been submitted for funding in the context of the EC's 6th Framework Programme in late 2003. This project will be the first attempt to develop a simple and robust *in vitro* testing strategy for predicting human acute oral systemic toxicity that could totally replace the animal tests currently used. The time necessary to achieve complete animal replacement is strongly dependent on the outcome of this project, and was estimated by the experts at no less than 10 years.

2. Skin Irritation / Corrosion

In the field of skin irritation, ECVAM is currently funding a validation study that if successful may result in three validated alternatives (the EpiDermTM human skin model, the EPISKINTM human skin model, and the mouse skin integrity function test (SIFT)) by 2004/2005, and EU regulatory acceptance by 2007/2008. These tests could enable full animal replacement for hazard identification purposes (classification and labelling). With regard to risk assessment (dose-response investigations), these methods might allow only reduction of animal experimentation. Other aspects such as reversibility and dose-response need to be addressed for achieving full animal replacement. It is estimated that this will represent a challenge that will keep scientists occupied beyond 2009.

In the field of skin corrosion, alternative methods have been already validated and accepted for regulatory use in the EU (Annex V B.40) and at OECD level (OECD TG 430 and TG 431) and no animal testing should be performed for this endpoint both for hazard identification and for risk assessment purposes.

3. Eye Irritation

Major validation and evaluation studies took place in the 90's to replace the Draize test for eye irritation testing. Although good reproducibility and reliability of the most valuable alternative methods was demonstrated, it was not possible to identify a single method able to replace the Draize rabbit eye test. This is due to different factors including the limited quality of the existing *in vivo* data, limitations of the animal test method, and the fact that the range of criteria for injury and inflammation covered by the Draize rabbit eye test is unlikely to be

replaced by a single *in vitro* test. The participating experts identified 14 advanced methods in the field, amongst which 6 were considered as most valuable. Four of these most valuable tests (i.e., the Bovine Corneal Opacity and Permeability (BCOP) test, the Isolated Rabbit Eye (IRE) test, the Chicken Enucleated Eye Test (CEET) and the Hen's egg test on the chorio-allantoic membrane (HET-CAM)) are already accepted by some European Member States Authorities for the classification of severe eye irritants although not formally validated. To achieve full replacement of the Draize rabbit eye test the experts recommended: (1) to assess the existing data by using a weight of evidence approach to validation, (2) to use a high quality *in vivo* data set, (3) to consider differences in the regulatory classification schemes, and the upcoming implementation of the GHS, (4) to use test strategies, (5) to define strategies for risk assessment purposes, and (6) to support the development of mechanistically-based models to find alternatives for the most difficult endpoints to be replaced such as reversibility or persistence. If these recommendations are followed and the tests prove to be valid, the most valuable methods could be validated and endorsed by ESAC for specific purposes within 4 years. Finally, the participating experts estimated as a compromise that more than 6 years would be necessary for achieving replacement of animal experimentation with regulatory accepted tests/strategies using the current EU classification systems.

4. Skin sensitisation

Two different, not mutually exclusive alternative approaches exist to assess the skin sensitising potential of chemicals and products: computer-based expert or QSAR systems and *in vitro* models. Among these, (Q)SARs systems are the most developed. It is envisaged that further optimisation of such methods could lead to validation and ESAC endorsement within the next 5-6 years. Evaluation criteria and guidance documents on the validation of these *in silico* systems are being defined by ECVAM and at OECD level. Concerning the cell-based systems, they have in some cases been shown to be capable of distinguishing between sensitisers and non-sensitiser (and could be used for priority setting), however they are still at a basic research level and may not achieve ESAC endorsement within the next 10 years. Further research and refinement activities for these *in vitro* models are still needed, and good coordination could help focus research on the development of predictive test methods. Due to the complexity of the mechanisms of skin sensitisation, a single test will not be able to replace the currently required animal experiments. Efforts will also be needed to identify the most relevant endpoints and to optimise the existing tests. However, the combination of several *in vitro* tests, which cover all relevant mechanistic steps of skin sensitisation, into a test battery

could lead to the replacement of the animal tests. There will be therefore the need to give guidance for the validation of test batteries. An indicative time foreseen for achieving validation of an alternative test battery for skin sensitisation is 10-12 years, 12-14 years being the time required for regulatory acceptance at the EU level.

5. Skin Absorption / Penetration

In the area of skin absorption/penetration, an alternative test is already accepted at the OECD level (TG 428) and by the SCCNFP although it has not passed through a formal prospective validation study. It can be considered as a full alternative to the *in vivo* test, although only limited experience with repeated-dose testing exists, and that the skin metabolism is not covered by the test. Further specific guidance for cosmetic ingredients is recommended for the standardisation of test protocols.

6. Subacute and subchronic toxicity

Chronic toxicity is a consequence of the persistent or progressively deteriorating dysfunction of cells, organs or multiple organ systems, resulting from long-term exposure to a chemical. Since a wide range of endpoints is investigated in the animal chronic toxicity studies, an integrated approach to repeated-dose toxicity testing based on the use of alternative methods with complementary endpoints needs to be developed. The present document presents the main available *in vitro* models in relation to five of the most common targets for toxicity (liver, kidney, central nervous system, lung and haematopoietic system). The identified tests are all at the research and development level, and none of them is seen today as an ideal method for the evaluation of any of the target organ toxicities. Efforts will be necessary to optimise the existing models, and to search for relevant *in vitro* models in those cases where fewer models are available (e.g., lung models). Additional basic research is also recommended to better understand the pathogenesis of chronic diseases in general. Finally, additional efforts are necessary to estimate the NOAEL *in vitro*, and more efforts and funding are needed to evaluate the application of QSAR approaches to predict chronic toxicity and to incorporate these tools into a testing battery and/or tiered strategy. In conclusion, the participating experts estimated that the validation of the existing models would need more than 10 years and that the time necessary to achieve full animal replacement with regulatory accepted tests/strategies will depend on the progress at the research and development level and adequate prioritisation, funding and coordination of efforts.

7. Genotoxicity and Mutagenicity

Although several *in vitro* tests are currently accepted by regulatory authorities to assess the genotoxic and/or mutagenic potential of a chemical, they present some limitations such as the lack of human-like metabolic capacity, toxicokinetics, oversensitivity compared to *in vivo* situations, or the use of cell lines not relevant to the target organs. For these reasons, the group of experts suggested a completely new test strategy, which is specifically tailored for the cosmetic industry. This test strategy is divided into 4 stages: stage 1 characterises the substance based on existing data and knowledge; stage 2 is a basic *in vitro* test battery for hazard identification; stage 3 is a follow-up stage using target *in vitro* model systems and is to be performed only if one or more tests are positive in stage 2; stage 4 uses animal testing and is to be performed only if one or more tests are positive in stage 3. Stage 1 is based mainly on existing information on the chemical. Stage 2 comprises regulatory accepted tests (Annex V B.13-B.14 or OECD TG 471; Annex V B.15 or OECD TG 480; Annex V B.17 or OECD TG 476; Annex V B.10 or OECD TG 473) and/or the optimised micronucleus *in vitro* test. Stage 3 comprises target *in vitro* models that still need to be developed and validated, in particular the participating experts recommended the use of skin cells or models as it is the first target for cosmetic products. Finally stage 4 would be performed only when necessary, and would comprise existing *in vivo* tests. The time estimated for implementation and validation of the building blocks of stage 3 is 8-10 years. To reach full replacement of animal tests, model systems in the area of toxicokinetics and metabolism are required. Moreover the emerging area of toxicogenomics could lead to a better understanding of the process of genotoxicity/mutagenicity which may help to develop the “right” *in vitro* models. Taking into account the state of the art in those areas, it was estimated that a total replacement of animal testing for genotoxicity/mutagenicity is not feasible within the next 12 years. The progress will depend on several factors such as the development of *in vitro* tests in the area of toxicokinetics and metabolism, the development of *in vitro* tests on target cells relevant for cosmetics, and further progress in the field of toxicogenomics. Importantly, the experts feel that better use of the flexibility provided in the current *in vivo* guideline approaches could result in a substantial reduction in animal use.

8. UV-induced toxic effects

No specific animal test is required for the UV-induced toxic effects. However, for cosmetic ingredients and mixtures of ingredients absorbing UV light (in particular UV filter chemicals used, e.g. to ensure light stability of cosmetics, or used in sun protection products) the acute

photo-toxicity (photo-irritation), photo-sensitisation and photo-genotoxicity potential needs to be assessed according to the SCCNFP “Notes for Guidance”.

In the area of acute phototoxicity an *in vitro* test (3T3 Neutral Red Uptake Phototoxicity Test or 3T3 NRU PT) has been already validated and adopted for regulatory use in the EU (Annex V B.41) and at the OECD level (OECD TG 432). It is regarded as a basic screen to identify acute phototoxic potential. Two additional tests, RBC phototoxicity test and human 3-D skin model *in vitro* phototoxicity test, that underwent validation and prevalidation respectively, are regarded useful and important adjunct tests to overcome some limitations of the 3T3 NRU PT, namely the fairly low UVB tolerance of the 3T3 fibroblasts and the inability to model the bioavailability of test materials topically applied to the skin. In addition, the Photo-RBC allows evaluation of the phototoxic mechanisms involved.

In the area of photo-genotoxicity, almost the whole battery of *in vitro* genetic toxicity tests has been (or is currently being) converted into test protocols of photo-genotoxicity tests. Currently, two tests (the Photo-Micronucleus Test and the Photo-Comet-Assay test) are being evaluated in a formal validation study. It is expected that these *in vitro* photogenotoxicity test methods may be validated and adopted at EU level within the next five years.

In the area of photo-allergy (-sensitisation), like in the area of development of predictive *in vitro* tests for delayed contact sensitisation (Allergenicity) potential without involvement of light, due to a lacking ability to model the complex mechanisms underlying allergy, currently no promising *in vitro* methods to predict photo-sensitisation potential are in sight (see chapter on skin sensitisation). The only promising alternatives currently under development are *in vivo* refinements, like the Photo Local Lymph Node Assay (LLNA). Once a reliable and predictive *in vitro* test battery and strategy for the assessment of “dark” sensitisation potential will be developed and accepted, adaptation into similar photo-sensitisation tests is considered possible.

9. Toxicokinetics and Metabolism

Toxicokinetics describes the kinetic processes of absorption, distribution, metabolism and excretion of a compound in an organism. The participating experts propose a completely new tiered approach based on *in vitro* and *in silico* models, comprising three steps: tier 1 assesses the likelihood to have systemic exposure, tier 2 determines the distribution of the compound, and tier 3 determines the potency of a compound. Only when a positive answer is obtained

with tier 1, there is the need to proceed to tier 2. The participating experts identified an inventory of the most valuable alternative tests available for tiers 1 and 2, whereas tier 3 comprises the alternative methods identified in the other subchapters. Tier 1 includes a battery of tests assessing dermal, oral and pulmonary absorption. The participating experts recommended further research and development efforts for alternative methods covering the inhalation route, as well as improvements in the intestinal barrier model. Tier 2 includes a battery of tests for the estimation of plasma level, excretion, bioaccumulation and biotransformation (metabolism). Within this tier, the need to further investigate and develop the excretion models (*in vitro* and *in silico*) was identified. Furthermore, the lack of appropriate tests to predict or measure the bio-accumulation of compounds was identified, and additional research and development in that area was recommended. The time necessary to achieve validation and ESAC endorsement of the identified methods for use in the context of cosmetics testing was estimated to be approximately 8 years for the completion of all the building blocks of tier 1, and of around 5 years for the relevant building blocks in tier 2. The experts estimated that more than 10 years would be required to achieve full replacement of the animal tests used for the evaluation of toxicokinetics/metabolism, due to the lack of tests for excretion which is however of less relevance for cosmetics as compared to other product classes. Therefore, without considering excretion, the estimated time would be 8 years. The progress that will be made in this area which is regarded as a bottleneck for the development of *in vitro* test strategies for regulatory use will depend on adequate prioritisation, funding and coordination of efforts. Additional recommendations made by the group are: (1) a follow-up of the discussions by organising an ECVAM workshop (which has taken place on January 26-29, 2004); (2) if biotransformation is an issue for a specific compound, this aspect should be considered in all 3 tiers of the test strategy; (3) the validation of the overall test strategy; (4) the importance of the selection of training or validation sets of chemicals; (5) and further research efforts to investigate the pulmonary exposure to particulate compounds.

10. Carcinogenicity

The carcinogenicity bioassays are rarely used by the cosmetic industries, but the bioassay is still needed to determine the potency and the target organ(s) of a carcinogenic compound. The process of carcinogenesis is the result from a sequence of stages and complex biological interaction that might be influenced by factors such as age, diet, environment, hormonal balance, etc. It is a long-term process, difficult to mimic with *in vitro* tests. The participating experts suggested that the carcinogenic potential could be detected by a combination of the identified existing *in vitro* tests. To achieve validation of these tests 5 years would be

necessary for the optimized tests, and more than 10 years for those under R&D. However, these tests present crucial limitations as the absence of metabolic capacity or the use of cell lines not relevant to predict the endpoint at target organ (see point 7). In addition, the identified tests might not predict and detect some non-genotoxic carcinogens since these act through a variety of mechanisms. The participating scientists stated therefore that the modelling of such complex adverse effects cannot be accomplished at present by non-animal tests, and they were unable to suggest a deadline for achieving the full replacement of animal tests.

11. Reproductive and Developmental Toxicity

Several *in vitro* tests were identified covering different biological components of the reproductive and developmental toxicity mammalian cycle. Three of the identified tests are already validated and endorsed by ESAC (the embryonic stem cell test for embryotoxicity, the micromass test for embryotoxicity and the whole embryo culture test for embryotoxicity). For the most optimised, but not yet validated, methods, the time to achieve ESAC endorsement was estimated to be 6 years, and for the tests at research level, the time was estimated to be 10 years. However, due to the complexity of the mammalian reproductive cycle, it is not possible to model the whole cycle with one single *in vitro* system. In addition, some areas were identified by the experts that cannot be replaced by the currently available *in vitro* assays such as reproductive behaviour, parturition and post-natal functional development. Finally, several of the proposed tests require the use of donor animals, and currently it cannot be foreseen if and when these tests can be replaced. A series of workshops was recommended to judge the relevance of the *in vitro* tests currently available and their potential role in a test strategy, to define the gaps of non-covered areas of the mammalian cycle, and to give guidance to the development of new tests that would be required. In addition, there is a proposal for an Integrated Project (ReProTect), which is under consideration in the context of the EC's 6th Framework Program, where the whole reproductive cycle was broken down into work packages and suitable tests were identified. These should now be evaluated, optimised, prevalidated and combined into a testing strategy. In parallel, the conceptual framework to compose and validate test strategies shall be developed.

Conclusions

The main success of the present exercise was to bring together a broad panel of stakeholders and 75 scientific experts representing industries, academia, animal welfare groups and governmental bodies that compiled their respective views on the topics discussed. The participating experts identified a number of tools currently known to be available that might contribute to the replacement of animal tests. Some human health effects can already today be assessed using alternative methods, i.e., skin corrosion, skin absorption and acute phototoxicity. However, for the data requirements in the other toxicological areas further efforts will be needed in order to achieve full replacement of the animal tests. If all the optimal conditions are met, the next areas where replacement of animal tests may occur are skin and eye irritation. In both areas advanced methods are currently available for, or undergoing, validation. Following in time, would be the area of toxicokinetics/metabolism regarded as a bottleneck for the development of *in vitro* test strategies for systemic toxicity. The gained information would allow further progression of the acute toxicity and genotoxicity/mutagenicity areas. If alternative methods were validated and adopted into regulation in these areas, they would lead already to an important reduction of animal use. Finally, the replacement of animal tests would take the longest in the areas of repeated dose toxicity, i.e., skin sensitisation, subacute/subchronic toxicity, carcinogenicity, and reproductive/developmental toxicity.

In general, the longer deadlines for phasing-out the animal experimentation were identified in those areas where the alternative methods are still under R&D, or where specific methods under R&D are required to complete the test strategies necessary to achieve full replacement of the animal tests. The necessity to have funds and human resources at R&D level is one of the major bottlenecks for obtaining alternative methods. However, good coordination and prioritisation to focus R&D and test optimisation efforts on alternative methods that are able to predict risks to human health are also crucial. Concerning those areas where the methods are already well advanced and ready for entering the (pre)validation process, the following needs were identified: the assessment of the existing data using weight of evidence approaches, the use of high quality *in vivo* data, the definition of criteria for validating test strategies as well as *in silico* models. As for R&D those efforts will only be possible if sufficient financial and human resources are made available. Furthermore, the implementation of the Globally Harmonised System (GHS) for classification could adversely impact timelines for regulatory acceptance.

The “ad-hoc Group” concluded that appropriate resources (human and financial) should be allocated to research and development (e.g., Industrial Programmes as well as National and European Funding Schemes). With regard to the validation process, ECVAM will also need to be correctly staffed and funded for being able to face the upcoming challenges. The level of funding and human resources provided at both, R&D and validation process, will affect the time needed to achieve full replacement of animal test. Finally, the improvement and optimisation of the regulatory acceptance procedure, either at Community level, or at OECD level, should be considered in order to make available replacement methods in a timely manner.

Summary Table

Human health effect	Valuable alternative methods identified	Time necessary to achieve validation (ESAC endorsement)*	Identified areas or endpoints with no alternative methods	Recommendations	Estimated time to achieve full animal replacement with methods adopted at the EU level*	Prospects
1 Acute toxicity	7 <i>in vitro</i> tests prevalidated or under (pre)validation for basal cytotoxicity (partial replacement/reduction) QSAR's models under R&D	2+ years for the 2 methods under validation 4+ years for the method under prevalidation	Metabolism Toxicokinetics Target organ toxicity for potentially sensitive target organ	Validation of testing battery Development of prediction model(s) Development of complementary endpoints	> 10 years	Progress will depend on the outcome of the A-Cute-Tox proposal for an Integrated Project submitted for funding in the context of the EC's 6 th Framework Programme. It is the first practical attempt to develop a simple and robust <i>in vitro</i> testing strategy for predicting human acute oral systemic toxicity that could totally replace the current animal tests
2 Skin corrosion	3 <i>in vitro</i> tests validated and adopted at EU and OECD level	0 years		Animal testing should not be performed for this endpoint	0 years	
2 Skin irritation	3 <i>in vitro</i> tests under validation, and 1 <i>in vitro</i> test optimised	1-2 years	For risk assessment: need to address reversibility and dose-response aspects	Develop test strategies for risk assessment purposes	4-5 years for hazard identification > 6 years for risk assessment	Hazard identification: implementation of the GHS could adversely impact timelines Risk assessment: requires further research
3 Eye irritation	14 <i>in vitro</i> tests optimised or under (pre)validation, amongst which 6 considered as most promising 1 refinement alternative	4 years for the most promising tests	Reversibility or persistence, inflammation, mechanical irritation, effects on conjunctiva	Assess the existing data using a weight of evidence validation approach Obtain a high quality <i>in vivo</i> data set Consider differences on the regulatory classification schemes, and the upcoming implementation of GHS Develop test strategies Support the development of mechanistically-based models Define strategies for risk assessment purposes	> 6 years	Current activities focus on the retrospective validation of the most promising methods

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4 Skin sensitisation	optimised <i>in silico</i> models, several <i>in vitro</i> models under R&D	5-6 years for (Q)SARs 5-12 years for <i>in vitro</i> tests		Further research in the identification of the relevant endpoints and further development-optimisation of existing <i>in vitro</i> tests Coordination of EU research activities on the development of predictive test methods to be incorporated in a test battery for the full replacement of the animal test Guidance for the validation of a test battery	12-14 years	
5 Skin absorption / penetration	1 <i>in vitro</i> test accepted at the OECD level and by the SCCNFP although not formally validated	0 years	Limited experience with repeated dosing Combination of skin absorption/penetration with metabolism is not covered by this test	Further guidance for cosmetic ingredients testing for a better standardisation of study protocols	6 months	
6 Subacute and subchronic toxicity	Several <i>in vitro</i> models under R&D for five of the most common targets for toxicity (liver, kidney, CNS, lung and haematopoietic system)	> 10 years	No generally accepted alternative available for replacing repeated-dose <i>in vivo</i> testing	Need of an integrated approach based on complementary endpoints Efforts necessary to optimise the existing models, and to identify adequate <i>in vitro</i> models where fewer robust models are currently available (e.g., lung models) Need of additional basic research to better understand the pathogenesis of chronic diseases Additional efforts necessary to estimate dose-response Evaluation of QSARs approaches to predict chronic toxicity and as a part of a testing battery and/or tiered strategy	n.d.	The progress made will depend on adequate prioritisation, funding and coordination of efforts

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7 Genotoxicity and mutagenicity	7 <i>in vitro</i> tests adopted at the EU and OECD level 2 <i>in vitro</i> optimised tests 3 <i>in vitro</i> tests under R&D	1-6 years for optimised tests >6 years for tests under R&D	Human-like metabolic capacity Toxicokinetics Oversensitivity compared to <i>in vivo</i> situations Cell lines not relevant to the target organs	Development of toxicokinetics and toxicogenomics Development of <i>in vitro</i> genotoxicity tests on target cell models relevant to cosmetics Leverage existing guidelines for <i>in vivo</i> testing to reduce current use of animals	> 12 years	The participating experts developed a new test strategy tailored for the cosmetic industry The experts felt that reduction of animals is an important tool not sufficiently utilized at the moment Full replacement will also depend on the progress in the field of toxicokinetics and toxicogenomics The progress made will depend on availability of funding and mobilisation of resources
8 Acute phototoxicity	1 <i>in vitro</i> test validated and accepted at the EU and OECD level and by the SCCNFP 2 <i>in vitro</i> tests that underwent (pre)validation	0 years	Fairly low UVB tolerance Inability to model the bioavailability of test materials topically applied to the skin	Use adjunct tests (that underwent (pre)validation) to overcome limitations	0 years	The prediction of acute phototoxic potential is without limitations possible with the <i>in vitro</i> tests currently available
8 Photo-genotoxicity	2 <i>in vitro</i> under validation 3 <i>in vitro</i> optimised tests	3 years for the two tests under validation	Toxicokinetics Cell lines not relevant to the target organs	Use 3D human skin models for photogenotoxicity testing to address at the same time toxicokinetics and the use of relevant target cells	5 years	It is hoped that with a sensitive test for photo clastogenicity potential plus the photo-comet test potential photocarcinogens can be detected
8 Photo-allergy (-sensitisation)	1 <i>in vivo</i> refinement method (P-LLNA)	like Skin Sensitisation plus 3 years for adaptation to photo-sensib. 8-15 years for <i>in vitro</i> tests		Once a valid <i>in vitro</i> approach (e.g. test battery) for skin sensitisation exists, adapt the tests to be performed with UVA-visible irradiation	> 15 years	Progress will depend on progress in the field of skin sensitisation

Human health effect	Valuable alternative methods identified	Time necessary to achieve validation (ESAC endorsement)*	Identified areas or endpoints with no alternative methods	Recommendations	Estimated time to achieve full animal replacement with methods adopted at the EU level*	Prospects
9 Toxicokinetics and metabolism	Several alternatives identified for tiers 1 and 2, whereas tier 3 comprises alternative methods described in the other subchapters	Tier 1: 3-8 years Tier 2: 3-5 years with one aspect (excretion) > 10 years	Tier 1: further R&D efforts for alternative methods dealing with the inhalation route, and improvements of the intestinal barrier models Tier 2: more R&D to predict or measure the bio-accumulation of compounds Further development of excretion models, although the panel regarded this information of less importance for cosmetics	ECVAM workshop which was held on January 26-29, 2004. If biotransformation is an issue for a specific compound this aspect should be considered in all 3 tiers of the test strategy Validation of the overall test strategy The importance of the selection of training or validation sets of chemicals Further research efforts to investigate the pulmonary exposure to particulate compounds	10 years (without considering excretion) > 12 years (if excretion is included)	The participating experts proposed a new tiered approach based on <i>in vitro</i> and <i>in silico</i> models The progress made will depend on adequate prioritisation, funding and coordination of efforts
10 Carcinogenicity	4 <i>in vitro</i> tests for detecting only the carcinogenic potential of a substance: 3 tests adopted at EU level 1 test under R&D	5 years for optimised tests > 10 years for method under R&D	Long-term process difficult to mimic with short-term <i>in vitro</i> tests		n.d.	The carcinogenicity bioassays are rarely used for cosmetics but the bioassay is still needed to determine potency and target organs of a carcinogenic compound <i>In vitro</i> models could detect the carcinogenic potential of a substance, however they present at present crucial limitations in order to achieve full replacement of animal tests <i>In vivo</i> assays with transgenic animals might allow for a reduction/refinement in animal use

Human health effect	Valuable alternative methods identified	Time necessary to achieve validation (ESAC endorsement)*	Identified areas or endpoints with no alternative methods	Recommendations	Estimated time to achieve full animal replacement with methods adopted at the EU level*	Prospects
11 Reproductive and developmental toxicity	3 validated <i>in vitro</i> tests for partial replacement several optimised or under (pre)validation <i>in vitro</i> tests several tests under R&D	Validated tests: 0 years Tests ready for validation: 6 years Tests under R&D: > 10 years Tests to be developed: ? years	Some areas identified which cannot be replaced by the currently available <i>in vitro</i> assays such as reproductive behaviour, parturition, post-natal functional development, etc Several of the proposed tests request the use of donor animals, and it is not currently foreseeable when and if these tests can be replaced	Development of test strategies Series of workshops to judge the relevance of different <i>in vitro</i> tests currently available and their potential role in a test strategy, define gaps of non-covered areas of the mammalian cycle, and give guidance to the development of new tests that would be required	n.d.	Progress will depend on the outcome of the workshop series and on the outcome of the ReProTect project which is an Integrated Project under consideration in the context of the EC's 6 th Framework Programme. With this project, the whole reproductive cycle was broken down into work packages and suitable tests were identified that now need to be evaluated, optimised, prevalidated and combined into a testing strategy. In parallel, the conceptual framework to compose and validate test strategies shall be developed

*Assuming optimal conditions