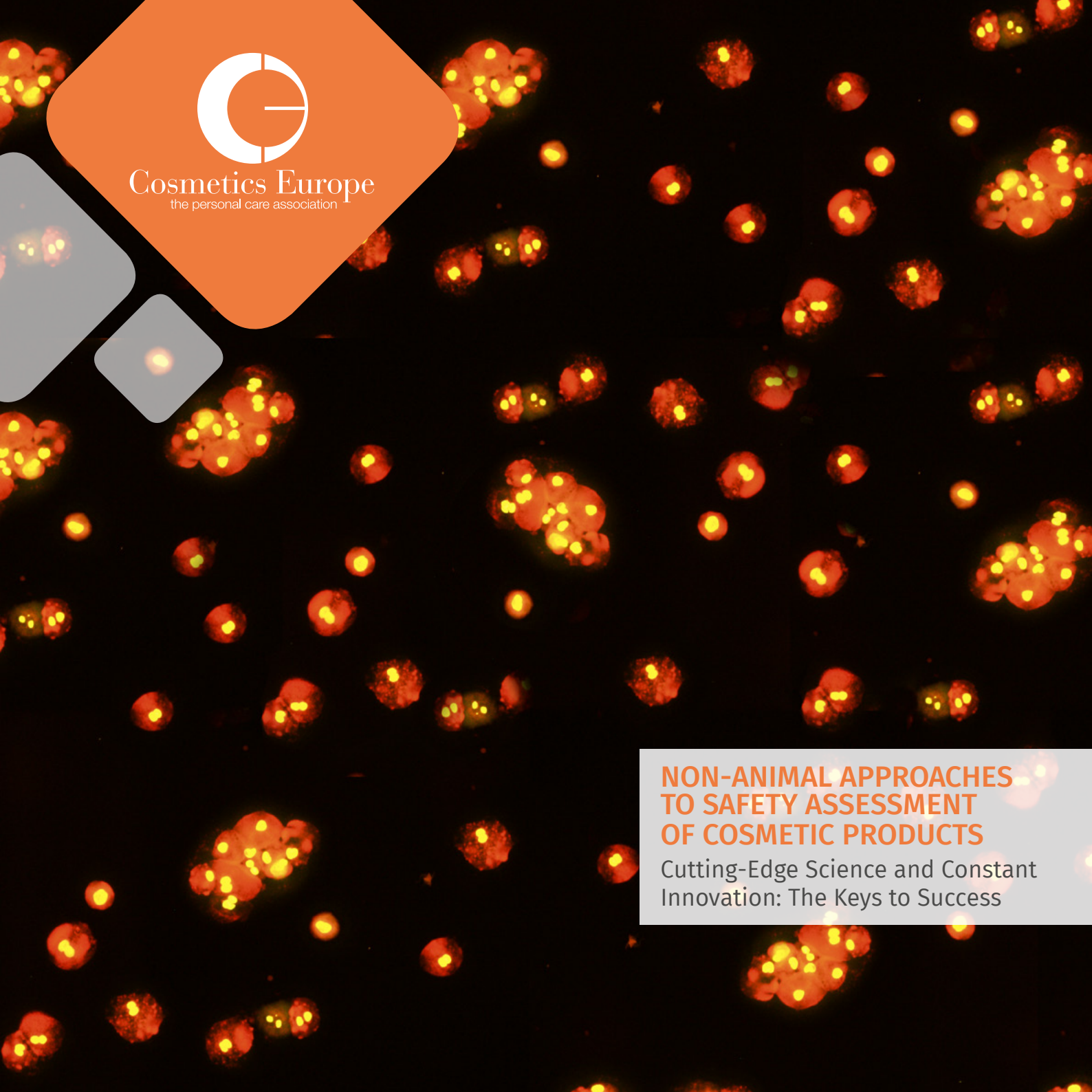

Cosmetics Europe
the personal care association
NON-ANIMAL APPROACHES TO SAFETY ASSESSMENT OF COSMETIC PRODUCTS
Cutting-Edge Science and Constant Innovation: The Keys to Success

Introduction

The safety of our products is the highest priority of the cosmetics industry, as well as a mandatory regulatory requirement. Safety assessments of our products, involving exhaustive testing of the ingredients they contain, and continuous improvement of testing and assessment capabilities, are therefore a constant focal point of our industry's research and innovation activities.

The EU Cosmetics Regulation governs how cosmetics and personal care products are made and placed on the market. It is the most comprehensive set of laws for our industry in the world, requiring cosmetics to be safe for human health when applied under normal or reasonably foreseeable conditions. To meet its obligations under the Regulation, our industry must:

- Run a highly comprehensive safety assessment
- Provide detailed product information
- Comply with ingredient and labelling rules

Both animal testing and the marketing of products containing ingredients tested on animals are subject to strict bans laid out in the Cosmetics Regulation. In order to perform a comprehensive safety assessment, the cosmetics industry must therefore rely on alternative methods to assess the suitability of ingredients, combined with the use of historical data.

An essential component of our research thus centres on the replacement of animal testing with alternatives to animal testing (AAT) when evaluating the safety of cosmetic products. While we fully support the goal of eliminating all unnecessary animal testing, we do have a major challenge on our hands: any new alternative approach for assessing safety must provide at least an equivalent level of consumer protection as the methods previously in place, and then go through a lengthy process to be accepted by the regulatory authorities.

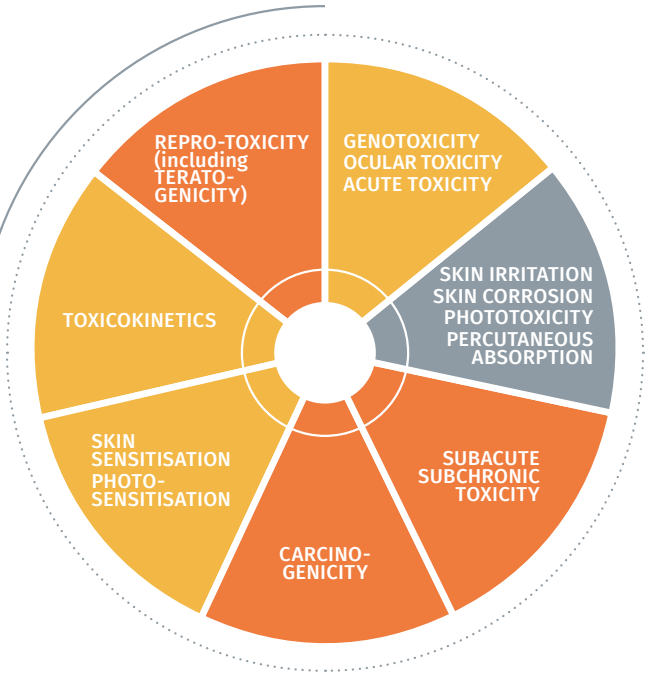
We do however have a head-start. The cosmetics industry has been at the forefront of developing AAT for more than 25 years. This sustained commitment, and the significant funds that have been invested in relevant science and research programmes, means we are now leaders in the AAT field. At Cosmetics Europe specifically, we have invested in several major initiatives, which we will lay out for you in this brochure, while outlining the major advances we have made and plans for the immediate future.

Status of AAT science: prospects by 2020

RESEARCH

ADVANCED

COMPLETED



Background: The Ban and Repercussions

Animal testing on ingredients for use in cosmetic products was banned in the EU in 2009. Given the unavailability of alternative methods for three endpoints (repeated dose toxicity, reproductive toxicity, and toxicokinetics) these were exempted from the testing ban until 2013. However, as of March 2013, the EU testing and marketing ban has covered all toxicological endpoints, irrespective of whether a full set of alternative methods is available to replace corresponding animal studies.

Despite considerable progress, alternative replacement test methods have not yet been developed or accepted by the international regulatory community for the safety assessment of ingredients for several endpoints (see pie chart). Until all toxicological endpoints can be covered by alternatives, we are severely limited in our ability to introduce new ingredients, apply existing ingredients for new uses, or respond to new questions regarding the safety of existing ingredients. And this is not a simple process whereby alternative test methods simply replace animal tests: integrated testing strategies are often required, employing a toolbox of alternative test methods to combine/gather enough data to adequately assess safety.

As a result, while we favour the elimination of unnecessary animal testing, we believe that in order to guarantee consumer safety and allow for innovation, any future ban on animal tests should be limited to toxicological endpoints for which validated alternative methods are already available.

The Long Range Science Strategy: Our Main Research and Science Programme

Our research into alternatives to animal testing is founded on multidisciplinary partnerships between Cosmetics Europe's member companies, and other groups that have a deep interest in alternative methods and approaches, including the international regulatory community, validating agencies, academia, research institutes, and industry partners (including large as well as small and medium sized enterprises).

Our main research programme, the Long Range Science Strategy (LRSS), is supported and funded by a consortium of Cosmetics Europe members. Started in 2016 and scheduled to run until 2020, it comprises a number of partners working together across the five most relevant areas for evaluating the safety of cosmetic ingredients: (i) eye irritation and severe eye damage, (ii) genotoxicity/mutagenicity, (iii) skin sensitisation, (iv) absorption, distribution, metabolism, and elimination (ADME), and (v) systemic toxicity. The data and outcomes generated in each of the five research areas have already allowed us to develop several robust safety assessment approaches based on alternative methods.

Programme pillars and goals

The goal of the LRSS is to enable animal-free safety assessment of cosmetic ingredients after repeated exposure, thereby entirely replacing repeat dose toxicity animal tests.

In order to meet this goal, the LRSS has three pillars:

1. Developing non-animal methods, testing strategies, and alternative approaches.

2. Using these alternative approaches and implementing them in a risk assessment paradigm, to show that safety assessments are possible on a broad spectrum of effects, with a focus on systemic toxicity.

3. Supporting the regulatory acceptance of these approaches and the data generated by applying them.

The LRSS intends to employ these pillars throughout several case studies. The following paragraphs explain the programme in further detail.

Testing for local effects of chemicals

In order to assess the safety of cosmetic products, our industry follows a strict scientific process as well as the regulatory requirements of all relevant authorities. Safety is generally assessed by examining the relevant toxicological endpoints of ingredients, and the likely local and/or systemic consumer exposure to the ingredient following likely usage. The main exposure route for cosmetic products is dermal and/or ocular. Topical application can thus potentially trigger various distinct local effects. We shall explore these and our work across each in the subsequent paragraphs.

Skin Irritation and Corrosion

Skin irritation and corrosion are two dermal local effects that are also respectively reversible and non-reversible. Cosmetics Europe has played a major role in developing numerous test methods that address these effects. Beyond individual test methods, various test method combinations are now common practice, and have been published by the Organisation for Economic Co-operation and Development (OECD) in its guidance documents on the Integrated Approach for Testing and Assessment (IATA)

Eye Irritation and Severe Eye Damage

The Cosmetics Europe Eye Irritation Programme focuses on the development and optimisation of alternative methods and models that evaluate the potential of a chemical to induce injury to the human eye. Its work covers eye irritation and severe eye damage: effects that are respectively reversible and non-reversible. The programme has delivered a full set of alternative methods, several of which are now OECD-accepted test guidelines and have been translated into European test method regulations. The focus is now shifting to how methods may be combined to design optimal testing strategies, thereby improving predictions across the whole range of ocular endpoints.

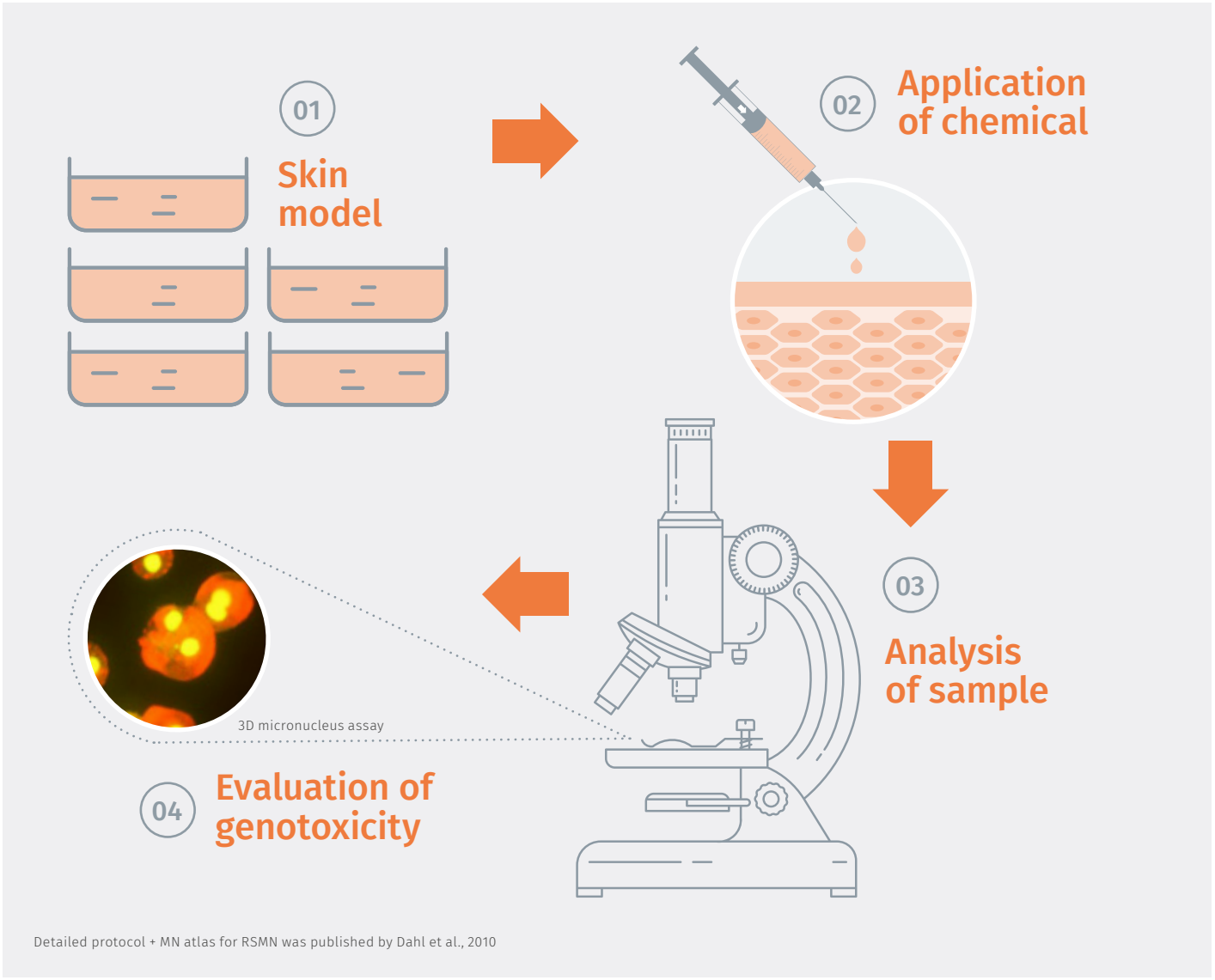
Genotoxicity

The Cosmetics Europe Genotoxicity-Mutagenicity Programme aims to develop new *in vitro* assays which are more relevant and predictive for the dermal route of exposure than the tests available today. Its current work focuses on developing assays that are based on reconstructed human skin tissues. By replicating human skin, these assays provide high quality predictions of the genotoxicity of a chemical via the dermal route of exposure. Specifically, 3D skin models are being used in two currently accepted tests, the Micronucleus and Comet assays, and have the potential to substantially improve their genotoxicity predictions. Based on testing results of a substantial number of model substances, the 3D assays are currently under evaluation for regulatory use and acceptance.

Skin Sensitisation

The Cosmetics Europe Skin Sensitisation Programme aims to develop a full set of *in vitro* methods that can be used to determine the ability of a substance to cause skin allergy. It has been collating all available information on how chemicals react with the skin and activate the body's immune system to cause skin allergy. The programme also focuses on biological parameters which represent potential key events in the induction of skin sensitisation in human, and is evaluating how the tests can be used in combination to best predict skin sensitisation potential.

Reconstructed skin models to test genotoxicity



Detailed protocol + MN atlas for RSMN was published by Dahl et al., 2010

Testing for systemic effects of chemicals

Systemic effects of chemicals relate to internal exposure i.e. when a chemical reaches the serum in sufficient quantities to trigger an effect at cell or organ level, irrespective of the route of exposure.

Systemic toxicity has two main components: kinetics, often called toxicokinetics (TK) , and toxicodynamics (TD). The former, TK, concerns interactions of the organism with the chemical and its fate: absorption, distribution, metabolism, and elimination. The latter, TD, results from the interactions of the chemical with the organism, its associated mechanisms, and toxicity effects.

Toxicokinetics: Absorption, Distribution, Metabolism, Excretion

Our work on skin bioavailability, metabolism, and potential systemic exposure aims to improve our understanding of how substances behave when applied to the skin. Specifically, our research addresses a multitude of toxicokinetic parameters to estimate internal dosage in relation to external exposure. This requires data on consumer exposure and the use of *in silico* and physiologically based pharmacokinetic (PBPK) models to help predict the systemic concentration of the chemical in relation to dermal exposure. The generation of *in vitro* ADME data to support *in silico* and PBPK models may also be needed at this stage. Our work will be split into several projects:

- Using a multi-organ chip, we will investigate the skin and liver-specific metabolism of chemicals after single and repeated dermal and systemic exposure.
- To assess topical exposure, we will determine how a chemical penetrates the skin and is then transformed by the skin by using measured skin bioavailability parameters and *in silico* tools.
- We will develop a toolbox of assays to measure *in vitro* ADME parameters that will help us to predict systemic exposure.
- We will develop an internal threshold of toxicological concern (TTC) using the TTC concept and the prediction of internal exposure.
- We will use a PBPK modelling specifically adapted for exposure to cosmetics, integrating ADME parameters and applying as proof of concept through case studies.
- We will use quantitative *in vitro – in vivo* extrapolation and *in vitro* kinetics to predict relevant concentrations of chemicals.

Toxicodynamics

The toxicodynamics project focuses on the toxicological mechanisms that may be triggered by exposure to a chemical, aiming to better understand molecular/cellular effects that cause adverse effects. The main components of the project are:

- A chemo-informatics platform will be developed in which we will collate toxicity data, identify *in silico* tools for analogue identification, property estimation and metabolite prediction, and utilise and capture mechanistic information and knowledge from Adverse Outcome Pathways with chemotypes.
- We will establish a repeated dose toxicity mode of action ontology to develop a structured system that links chemical features, modes of action, and repeated dose toxicity effects.
- A toolbox of toxicodynamic assays will be built. The selected TD assays will be driven by case study needs and will be evaluated in light of their reliability and relevance.

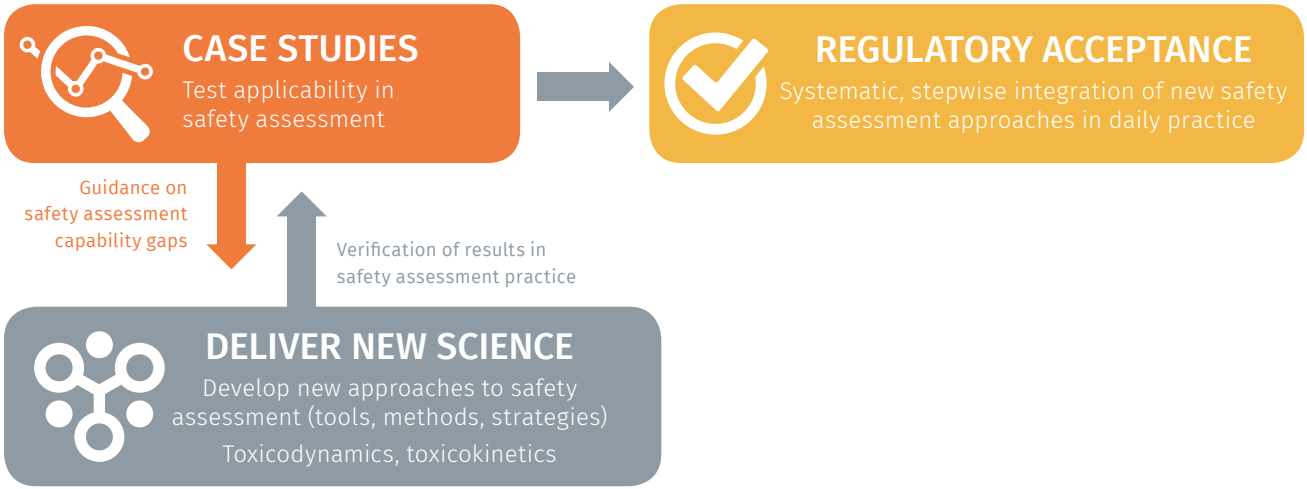
The toxicodynamic and toxicokinetic projects provide complementary information, and a combination of their methodologies will help us build new approaches to safety assessment.

Bringing the pieces of the puzzle together

Our knowledge of the biological mechanisms that cause toxicity has evolved substantially over the last five years, and the sequence of events that occur when a substance causes a negative effect on an organism can now be represented in a schematic way by adverse outcome pathways. However, *in vitro* assays usually recapitulate only some of these events, meaning that the knowledge provided by several non-animal methods must be combined to form alternative approaches or testing strategies. The integrated approach to testing and assessment (IATA) concept goes a step further by also considering existing data, and the option of performing read-across and weight-of-evidence, and analysing physico-chemical properties.

When performing a risk assessment, we integrate all available knowledge, including information on expected exposure to a certain chemical, knowledge of similar substances, data gathered using non-animal methods, and approaches for topical and systemic endpoints. The combination of these sources, in addition to the toxicological expertise of the safety assessor, help us meet the stringent safety requirements that remain our industry's key priority.

Case studies play an essential role in the LRSS programmes, driving practical implementation of scientific workflows and providing a proof of principle for safety assessment exclusively based on non-animal data, by integrating data from across all LRSS projects.



Collaboration and Partnerships

Research into alternatives to animal testing is complex: advancement will only be possible through multidisciplinary cooperation. Beyond the LRSS, Cosmetics Europe has collaborated on several programmes with a wide range of stakeholders in the field of alternatives to animal testing, and continues to do so.

EU level initiatives support transnational and cross-sector cooperation, in particular through joint agenda setting, mobilisation of additional funding and increased leveraging of industrial R&D investment, mainly with the European Commission and other partners under the Horizon 2020 programme. Key programmes which Cosmetics Europe has partnered on include:

- The SEURAT-1 programme from 2010-2015, which focused on systemic toxicity. The project was the largest ever private-public initiative in the field of AAT. Partnering with the European Commission, our industry invested €25 million of the total €50 million project budget. SEURAT-1 brought together over 70 universities, research institutes and companies with the aim to develop a consistent research strategy for alternative safety assessment of chemicals. This included establishing innovative animal-free toxicity testing methods for a better understanding of repeated dose toxicity and identifying gaps in knowledge that were to be bridged by future research work.
- EU-ToxRisk (2016-2020) is an international consortium funded by the European Commission. It comprises 39 partner organisations, including Cosmetics Europe, who together work on developing new concepts in regulatory chemical safety assessment, aiming to deliver reliable, animal-free hazard and risk assessment of chemicals. The EU-ToxRisk programme is looking to become the European flagship for animal-free chemical safety assessment. It builds on testing strategies and knowledge developed in previous national and European projects, including the SEURAT-1 programme.

Constant dialogue and collaboration with regulatory stakeholders such as the OECD, the EU institutions, and in particular their scientific committees and the European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM), will help to ensure that newly developed methods and approaches can be applied in a regulatory setting.

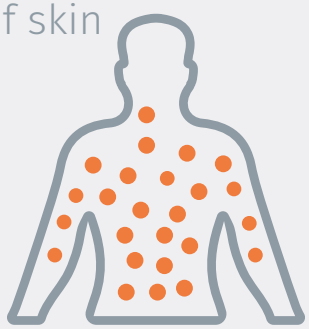
THE COSMETICS INDUSTRY HAS BEEN AT THE FOREFRONT OF DEVELOPING AAT FOR MORE THAN 25 YEARS.

Validation of 11 methods for 4 toxicological effects, applicable across industries

In vitro method for diffusion through the skin

Validation for *in vitro* eye irritation tests

Pre-validation of skin allergy methods



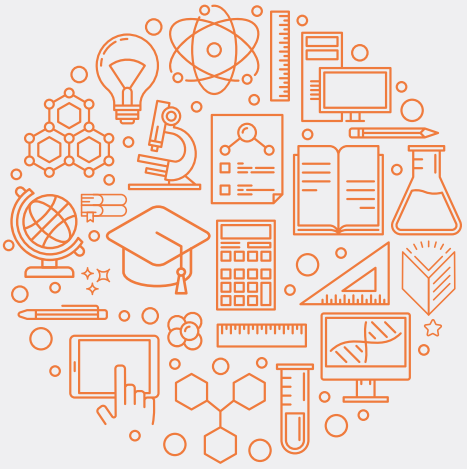
€44 Million
spending
2007-2015

€20 Million
LRSS
2016-2020

Representing approx.

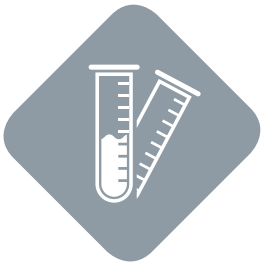
4000 companies

Over 200
scientific publications & presentations since 2007



Collaboration with more than **40 universities** and **research organisations**

We personally care



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For any information, please contact us at:
cosmeticseurope@cosmeticseurope.eu
www.cosmeticseurope.eu