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

2023 EDITION

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 their ingredients, and continuous improvement of testing and assessment capabilities, are therefore a constant focal point of our industry's research and innovation activities.

The European Union (EU) Cosmetic Products Regulation (EC 1223/2009) governs how cosmetics and personal care products are produced 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 EU Cosmetic Products Regulation. More and more countries outside the EU are following this example and introducing similar measures. In order to perform a comprehensive safety assessment, the cosmetics industry must therefore rely on robust alternative methods to assess the suitability of ingredients, combined with the use of historical data.

As we fully support the goal of eliminating all unnecessary animal testing, an essential component of our research is focused on the replacement of animal testing with alternatives to animal testing (AAT) when evaluating the safety of cosmetic ingredients and products. Any new approach methodology (NAM) or next generation risk assessment (NGRA) approach applied to assess safety, must provide at least an equivalent level of consumer protection as the methods/approaches previously in place, and then go through a rigorous process to be accepted by regulatory authorities.

Our sector has been at the forefront of developing AAT for more than 30 years. This sustained commitment, and the significant funds invested in relevant science and research programmes, have made us now leaders in the AAT field. At Cosmetics Europe, we have invested in several major initiatives. This brochure describes these initiatives, and outlines the major advances made and plans for the immediate future.

THE COSMETICS INDUSTRY HAS BEEN AT THE FOREFRONT OF DEVELOPING AAT FOR MORE THAN 30 YEARS.

Background: The Ban and Repercussions

Testing ingredients on animals for use in cosmetic products was banned in the EU in 2009. At that time, lack of suitable alternative methods for three endpoints (repeated dose toxicity, reproductive toxicity, and toxicokinetics) was recognised and therefore 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. Many countries aim to follow the EU example and enact a ban.

Over the years, there has been considerable progress in developing animal testing alternatives. Step by step, new approaches to ingredient safety assessment are being accepted by the regulatory community. However, more work still needs to be done.

Worldwide there is an ever-increasing desire to bring safe products to market without animal testing, which requires a new approach to how safety is assessed. The International Cooperation on Cosmetics Regulation (ICCR) requested for a group of scientists from regulatory authorities and the cosmetics industry to define the principles to integrate NAMs into risk assessments for cosmetic ingredients.[1,2] This group determined:

- the overall goals of NGRA: to be human-relevant, exposure-led, hypothesis-driven and designed to prevent harm;
- how an NGRA should be conducted: i.e. 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; how the assessment should be documented: transparent and explicit about the logic of the approach and sources of uncertainty.

1. Dent et al., Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients. Computational Toxicology, 7, 2018; pp. 20-26.
2. International Cooperation on Cosmetics Regulation (ICCR). Integrated Strategies for Safety Assessment of Cosmetic Ingredients - Part 2. Available online: https://www.iccr-cosmetics.org/downloads/topics/iccr_integrated_strategies_for_safety_assessment_of_cosmetic_ingredients_part_2.pdf. Last accessed 11 August 2021.

The Long Range Science Strategy:

Our Main Research and Science Programme

Our research into alternative approaches to animal testing was founded on multidisciplinary partnerships between cosmetics companies and other groups that have a deep interest in NAMs and NGRA, including the international regulatory community, validating agencies, academia, research institutes and industry partners (including large, small and medium-sized enterprises).

The Long Range Science Strategy (LRSS) programme has been supported and funded by a consortium of Cosmetics Europe members. The LRSS programme started in 2016 and has ended in 2022.

€17 Million
invested in the LRSS programme 2016-2022

It comprised a number of partners working together across seven areas most relevant for evaluating the safety of cosmetic ingredients:

- Eye irritation and severe eye damage
- Genotoxicity/mutagenicity
- Skin sensitisation
- Inhalation
- Exposure
- Toxicokinetics (absorption, distribution, metabolism and elimination (ADME))
- Toxicodynamics

The data and outcomes generated in each of the seven research areas have already allowed us to develop several robust safety assessment approaches based on alternative methods.

Since 2020, the LRSS programme also started covering a series of environmental research activities. Projects on SPERCs (Specific Environmental Release Categories), and marine exposure modelling are integrated into the regulatory acceptance objectives of the Cosmetics Europe LRSS Programme.

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LRSS programme goal and pillars

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

In order to meet this goal, the LRSS had three pillars.

1. Filling critical science gaps, for example related to specific endpoints or to mechanistic understanding and interpretation of NAMs.
2. Using NAMs and implementing them in NGRAs to show that safety assessments are possible on a broad spectrum of effects, with a focus on systemic toxicity.
3. Supporting wide uptake of NAMs and NGRA in the industry, and regulatory acceptance of these approaches and the data generated by applying them.

The NAMs used in safety assessment are at different stages of implementation, whereby some are already routinely used, while others require more evidence to support their use (Figure 1).

Figure 1: Status of NAMs for use in the risk assessment of cosmetic ingredients*

Already in use in cosmetic risk assessment	Mature technology with likely utility in cosmetic risk assessment	May have utility but insufficiently developed for current use
Read across	'Omics (especially transcriptomics)	Organ on chip
Exposure based waiving	In vitro pharmacological profiling	
In silico tools	Pathways modelling	
Metabolism and metabolite identification	3D culture systems (for systemic effects)	
PBPK modelling		
In chemico assays		
Reporter gene assays		
3D culture systems (local effects and genetic toxicity) Human studies		

The LRSS employed these pillars throughout several case studies developed to facilitate dialogue with stakeholders and to support safety dossiers for specific ingredients.

These case studies range from high-throughput evaluation using multiple toxicodynamic assays for the purpose of priority-setting, to in vitro or in vivo extrapolation for specific activities, and even the use of dietary comparators to put in vitro mode-of-action data into context.

Several Cosmetics Europe LRSS case studies have already been published by the Organisation for Economic Co-operation and Development (OECD).

*Figure adapted from: International Cooperation on Cosmetics Regulation (ICCR). Integrated Strategies for Safety Assessment of Cosmetic Ingredients - Part 2. Available online: https://www.iccr-cosmetics.org/downloads/topics/iccr_integrated_strategies_for_safety_assessment_of_cosmetic_ingredients_part_2.pdf. Last accessed 11 August 2021.

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. 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 usage. The main exposure route for cosmetic products is dermal and/or ocular, but for some types of consumer products, inhalation is also a considered exposure route where 'portal of entry' or 'local effects' can be seen. Exposure via all three routes can potentially trigger various distinct local effects. Therefore, we are exploring 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 reversible and non- reversible respectively. 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 OECD in its guidance documents on the Integrated Approach for Testing and Assessment (IATA).

Eye Irritation and Severe Eye Damage

The Cosmetics Europe LRSS Eye Irritation Programme focused on the development and optimisation of defined approaches that evaluate the potential of a chemical to induce injury to the human eye. The work covers eye irritation and severe eye damage: effects that are reversible and non-reversible respectively.

The programme has delivered two defined approaches for non- surfactant liquids, which have now been adopted by the OECD in a test guidelines. An OECD case study demonstrating the practical application of the defined approaches for liquids has been accepted. The focus is now on expanding the applicability domain to solids and surfactants thereby improving predictions across the entire range of UN Globally Harmonized System of Classification and Labelling of Chemicals (GHS) categories. In that context, defined approaches have been developed for surfactants and are under development for solids; these are currently being evaluated for regulatory use and acceptance by the OECD.

Genotoxicity/Mutagenicity

The Cosmetics Europe LRSS Genotoxicity Programme has developed and validated new in vitro assays based on reconstructed human skin tissues to mimic dermal exposure. The Micronucleus and Comet assays were adapted for use with 3D skin models and have been demonstrated to substantially improve genotoxicity predictions, and can serve as a direct replacement of in vivo genotoxicity follow-up tests to positive results from the standard in vitro testing battery. Based on the outcome of an international validation study led and funded by LRSS, both 3D skin assays have been accepted for OECD guideline development and are currently undergoing formal validation peer review. The 3D skin micronucleus assay is also being adapted to assess photogenotoxicity, an endpoint for which a suitable assay is currently lacking but is required as per the EU Scientific Committee on Consumer Safety (SCCS) Notes of Guidance.

Parallel work has evaluated how metabolic supplements to standard in vitro genotoxicity assays can better reflect Phase 1 and 2 pathways in the liver and skin. The aim is to reduce the rate of misleading or false positive results and to provide a model which incorporates relevant and organ-specific metabolism.

Skin Sensitisation

The Cosmetics Europe LRSS Skin Sensitisation Programme aims to develop a full set of NAMs 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 NAMs can be used in combination (via defined approaches) to best predict skin sensitisation potential. An NGRA framework for skin sensitisation has been developed and published, and is included in the EU Scientific Committee on Consumer Safety (SCCS) Notes of Guidance. It is being tested in case studies to show that safety assessments are possible on a broad range of applications and chemistries.

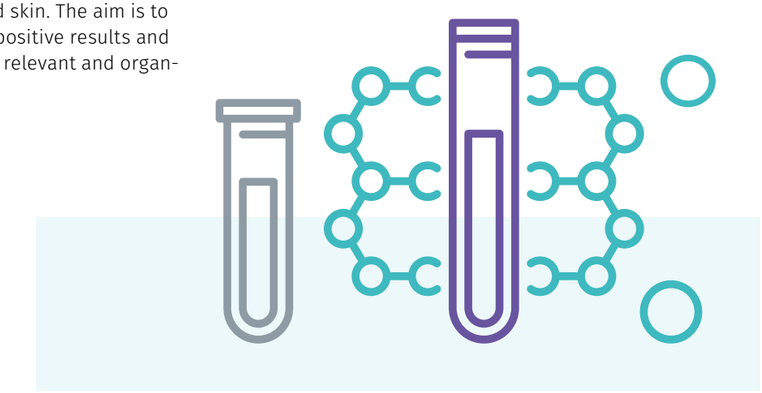
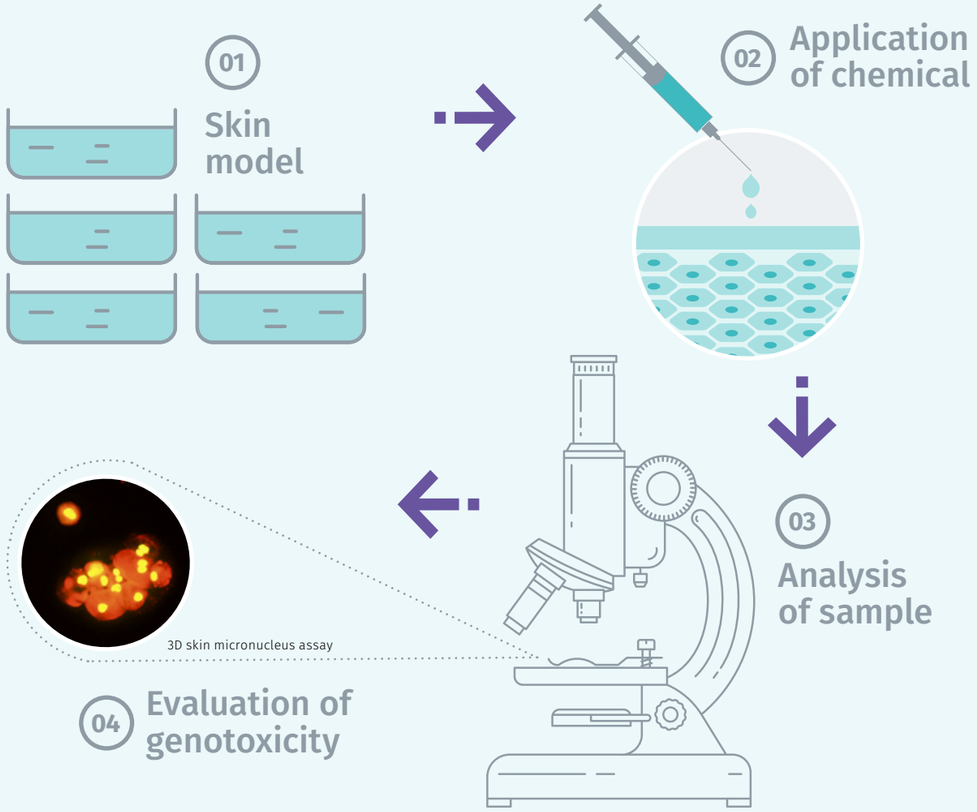


Figure 2: Reconstructed skin models to test genotoxicity



Dahl et al., The reconstructed skin micronucleus assay (RSMN) in EpiDerm™: detailed protocol and harmonized scoring atlas. Mutat Res. 2011;720(1-2):42-52.

Testing for systemic effects of chemicals

Systemic effects of chemicals relate to internal exposure, i.e. when a chemical reaches the blood 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, and toxicodynamics. Toxicokinetics concerns the fate of a substance: absorption, distribution, metabolism and elimination. Toxicodynamic effects result from the interactions of the substance with the organism, its associated mechanisms and potential toxicity effects.

Toxicokinetics: Absorption, Distribution, Metabolism, Excretion

Cosmetics Europe LRSS's work in the field of toxicokinetics aims to improve our understanding of the fate of substances when applied to the skin (since the major route of exposure to cosmetic ingredients is dermal). Specifically, our research addresses a multitude of toxicokinetic parameters to estimate internal concentrations in relation to external exposure. This requires the use of in silico, in vitro and physiologically based pharmacokinetic (PBPK) models to help predict the systemic concentration of the substance and possible metabolites in relation to dermal exposure, and to relate these to toxicodynamic findings. The generation of in vitro ADME data are needed to support in silico and PBPK models.

Our work is split into several branches:

- Use of a multi-organ microphysiological chip model to investigate the combined skin- and liver-specific metabolism of chemicals after single and repeated dermal and systemic exposure. Extension of the model to include a tissue to represent a target organ for endocrine disruption, the thyroid.
- Determining how a chemical penetrates the skin, measuring skin bioavailability parameters and developing in silico penetration tools to assess topical exposure.
- Developing a toolbox with methods to measure in vitro ADME parameters to help with prediction of systemic exposure.
- Developing an "internal threshold of toxicological concern" (iTTC) approach based on the TTC concept. To derive iTTC values, generation of ADME data for use in PBPK models is needed to estimate the internal exposure to iTTC chemicals.
- Using PBPK modelling specifically adapted for exposure to cosmetics, integrating ADME parameters and applying this modelling as proof of concept using case studies.
- Measurement of in vitro biokinetics to help interpret toxicodynamic effects in assays, and predict relevant concentrations of chemicals.

Toxicodynamics

Cosmetics Europe LRSS Toxicodynamics' project focuses on the toxicological mechanisms that may be triggered by exposure to a chemical, aiming to better understand molecular/ cellular effects that may cause adverse effects. The project has three main components:

- A chemoinformatics platform is under development to collate toxicity data, identify in silico tools for analogue identification, property estimation and metabolite prediction, and to utilise mechanistic information.
- A repeated dose toxicity ontology model has been developed and cosmetics-relevant compounds have been tested.
- A toolbox for toxicodynamic effects (including cell stress assays, in vitro pharmacology profiling and toxicogenomics) has been developed and used in 16 case studies. These include read-across and ab initio assessment using NAMs. To date, there have been three case studies endorsed and/ or published by the OECD as part of the OECD IATA Case Studies Project: parabens, caffeine and phenoxyethanol (OECD, 2020a; OECD, 2020b; OECD, 2020c). The experimental phase of the remaining case studies is now complete and the case studies will be published in due course.

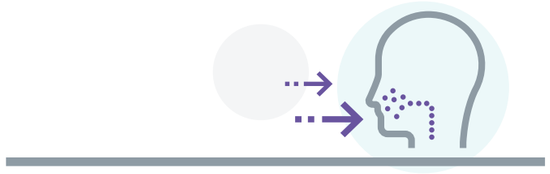
The toxicokinetic and toxicodynamic projects provide complementary information, and a combination of their methodologies will help us build new approaches to safety assessment.

Other areas of focus

Inhalation

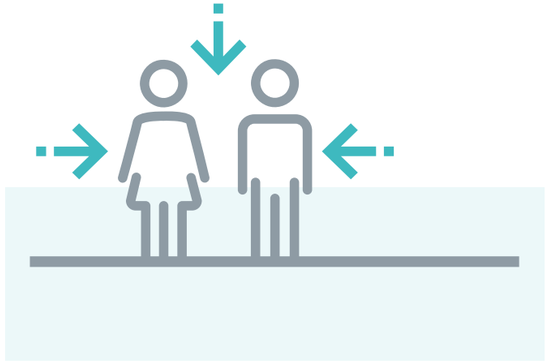
The Cosmetics Europe LRSS Inhalation Programme aimed to develop industry best practice and enhance confidence in the application of NAMs for assessment of inhalation exposure and toxicity safety. Its work has included interaction with regulators with regards to the inhalation TTC approach. While inhalation TTC has been acknowledged by regulators, it does not yet have their full support. The industry is therefore working to improve the methods. An online workshop was organised in 2020 to discuss the current state of the science and evaluate the robustness of a proposed revised approach. The workshop findings have been submitted for publication to accelerate regulatory and broader scientific acceptance of the approach. Moreover, guided by the outputs of the workshop, a project in collaboration with RIFM, P&G, US EPA, Fraunhofer ITEM is ongoing with the main purpose being to combine the inhalation exposure datasets and harmonize the data to enhance the existing TTC approaches.

To increase the robustness and regulatory acceptance of the available in silico models used for the determination of inhalation exposure assessments, this programme also aimed to harmonise and justify the parameters used in these simple computational/mathematical exposure models. A publication is in production regarding a harmonised approach to the parameterisation of in silico models. These in silico tools are an important part of the exposure-led NGRA approach for cosmetic products with unintentional exposure via the inhaled route.



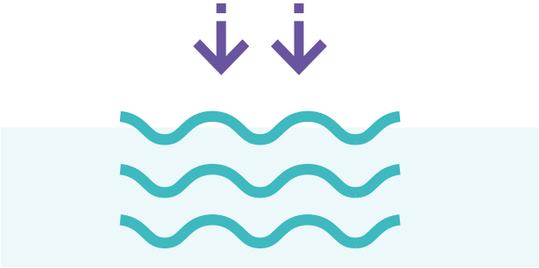
Exposure

The Cosmetics Europe LRSS Exposure Programme focuses on the development of tools and production of data required for realistic estimates of consumer exposure. In particular, the Aggregate Exposure project aimed to provide useful data to regulatory bodies for ingredients evaluation and assessment, including aggregate exposure. The programme also included work to generate exposure data in relation to infants aged 0-3 years. A web application has been developed for a Probabilistic Aggregate Consumer Exposure Model (PACEM tool). This will provide industry and stakeholders with a free and user-friendly exposure model tool.



Environment

The Cosmetics Europe LRSS environmental research projects focus on updating SPERC documentations for cosmetic products and marine exposure modelling. SPERCs provide more realistic and refined emission scenarios for REACH registration risk assessments (compared to default emission scenarios). It includes release factors for chemicals and efficiencies of risk management measures/ operation conditions in reducing chemicals emissions.



The persistence project focuses on developing a standardized weight of evidence approach to determine chemical persistence in a robust and reliable way.

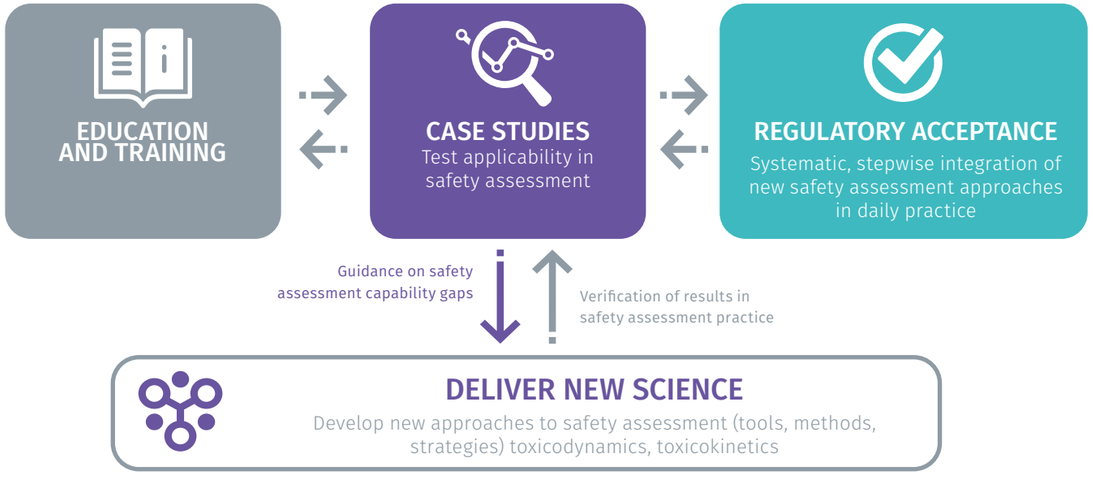
The marine exposure modelling project focuses on developing a framework to assess the environmental exposure of ultraviolet filters in freshwater and marine systems. Both environmental projects are closely integrated into the regulatory acceptance objectives of the Cosmetics Europe LRSS programme.

Bringing the pieces of the puzzle together

Our knowledge of the biological mechanisms that cause toxicity has evolved substantially over the last ten years. 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, a NAM usually covers only some of these events, meaning that the knowledge provided by several NAMs must be combined to form alternative testing strategies. The NGRA 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, which remains our industry's key priority.

Case studies played a key role in the Cosmetics Europe LRSS programme, 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. The LRSS initiated 16 case studies to demonstrate the applicability of NGRA approaches and NAMs. Case studies are employed in different ways – they can be used to engage with internal and external stakeholders to build confidence and trust in the new approaches and they can be used to strengthen the safety assessment of critical ingredients.



Collaboration, Partnerships and the Future

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. Cosmetics Europe has been a partner in several key projects.

- 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 alternatives to animal testing. 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 to be bridged by future research work.
- EU-ToxRisk (2016-2020) — an international consortium funded by the European Commission. It comprised 39 partner organisations, including Cosmetics Europe, who together worked 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 was the European flagship initiative for animal-free chemical safety assessment. It built on testing strategies and knowledge developed in previous national and European projects, including the SEURAT-1 programme.

Cosmetics Europe is currently involved in three new publicly funded projects on alternatives to animal testing, representing a total value of over 40 million Euros.

- The Virtual Human Platform for Safety Assessment (VHP4Safety) is an ambitious project to develop the world's first virtual human platform to determine the safety of chemicals and pharmaceuticals for human health based solely on human biology. By integrating innovations in data science, human tissue culture models and transition management, its aim is to accelerate the transition to animal-free safety assessment.
- RISK-HUNT3R, the successor of EU-ToxRisk, aims to develop a reliable, efficient, and cost-effective chemical safety assessment approach. It will be based entirely on non-animal methods and provide improved protection of the human population against the systemic health effects caused by (chronic) chemical exposure.
- ONTOX builds on the joint Cosmetics Europe/CEFIC (European Chemical Industry Council) ontology project on systemic toxicity. The vision of the ONTOX consortium is to provide a functional and sustainable solution for advancing human risk assessment of chemicals without use of animals, in line with the principles of 21st century toxicity testing and NGRA.

RISK-HUNT3R and ONTOX are funded under the Horizon Europe Programme and, together with PreisionTox, are part of the ASPIS cluster.

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.

What is next?

The global International Collaboration on Cosmetics Safety was founded in 2022 to advance the acceptance of animal free science worldwide.

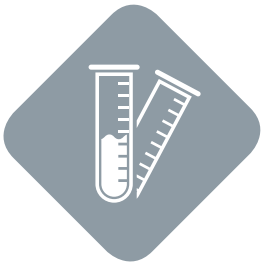
This global collaboration of cosmetics and chemicals industry experts will work together with animal protection NGOs to accelerate widespread use of animal-free safety science through research, education and regulatory engagement.

ICCS will collaborate with existing organizations to:

- Evaluate and further develop animal-free safety assessment approaches and demonstrate their scientific validity for human health and environmental protection.
- Share the results of these evaluation activities with regulators to inform ongoing regulatory acceptance discussions around the world.
- Provide education and training materials to accelerate widespread adoption of the latest animal-free safety science.

For more details on ICCS and its members, visit **www.iccs-cosmetics.org**.

We personally care



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