

ADVANCES IN GENOTOXICITY TESTING: A KEYSTONE OF OUR WORK ON ALTERNATIVES



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Welcome to the Cosmetics Europe research newsletter, in which we update our members and other stakeholders on our Long Range Science Strategy (LRSS) programme, which focuses exclusively on alternatives to animal testing. In this second edition, we look at the crucial topic of genotoxicity. As ever, I hope the newsletter will spark dialogue, and urge you to get in touch with me or any of my colleagues if you have questions or comments.

Our industry's finest scientists are at the forefront of developing alternatives to animal testing (AAT) for the safety assessment of cosmetics ingredients. Their work is more pressing than ever: the full EU ban on products containing ingredients tested on animals, in place since 2013, means we must rely entirely on alternatives.

In light of this, we launched the The Long Range Science Strategy (LRSS) in 2016, a multi-stakeholder programme that builds on our 20 years of experience in AAT. Currently the main research priority at Cosmetics Europe, the many partners involved in LRSS share three goals: developing effective alternative methods

and testing strategies; providing proof points to demonstrate that safety assessments using AAT are effective; and ensuring regulatory acceptance.

In this edition, we will explore an essential component of AAT: genotoxicity, which represents one of the five main work-streams of the LRSS. Our genotoxicity task force, led by Stefan Pfuhrer since its initiation in 2004, has conducted essential work in two core areas where genotoxicity testing approaches have faced challenges with regards to alternative methods: low predictive capacity of the established *in vitro* testing battery, and the need for higher tier assays to follow-up on non-concordant data.

In the rest of the newsletter, Stefan will outline the scope and activities of his task force in more detail, focusing on how the team has – and continues to – address the key challenges of genotoxicity testing in a post animal-testing world. Lastly, we include the expert opinion of Dr. Rodger Curren, a genotoxicity specialist and CEO at Institute for *In Vitro* Sciences, who collaborated in the development of genotoxicity methods.

I very much hope you will enjoy the newsletter, and look forward to engaging further with you on these issues, which are so essential to the future of our industry.

THE GENOTOXICITY TASK FORCE: SCOPE, ACTIVITIES AND NEXT STEPS

Dr. Stefan Pfuhrer
Chair, Cosmetics Europe Genotoxicity Task Force

Genotoxicity refers to processes which damage DNA. Testing for genotoxicity is an important building block to ensure the safety of cosmetics ingredients, thereby avoiding DNA damage and its potential undesired health consequences like genetic defects and cancer.

Given that the EU Cosmetics Directive does not permit animal testing for cosmetics under any circumstances, it is now no longer possible to conduct *in vivo* tests for cosmetic ingredients, including confirmatory genotoxicity tests. As John alluded to in his introduction, this presents a real challenge. As a result, the Genotoxicity Task Force has focussed on two areas in particular: (1) the low predictive

capacity of the standard *in vitro* genotoxicity battery, which despite being highly sensitive, leads to numerous so-called 'false positives'; (2) the need for higher tier assays to enable follow-up on non-concordant data, with a focus on the skin, the main route of exposure for cosmetic ingredients.



OUR WORK SO FAR

We have collaborated with prominent partners across the globe and discussed our strategy with the European Union Reference Laboratory for Alternatives to Animal Testing (EURL-ECVAM), the most important validation body for our industry, and scientific panels such as the Scientific Committee for Consumer Safety (SCCS), which advises the European Commission on health and safety risks. We are pleased with the progress we have achieved so far in both focus areas.

The so-called false positives issue means that the current standard battery of tests may lead to the incorrect labelling of ingredients as potential rodent carcinogens, and thus eliminate their potential use in cosmetic products. Over the course of several years, we conducted a project aimed at improving the predictive capacity of mammalian cell based genotoxicity assays which suffer from a high rate of false positives. We were advised by scientists from EURL-ECVAM in critical phases, such as chemical selection. The results of this project indicate that by using cells of human origin and a careful selection of

cytotoxicity criteria we can achieve a 2/3 reduction in the rate of false positives. This means that without compromising our high safety standards, fewer promising ingredients will unnecessarily be eliminated in the future.

Since animal testing can no longer be used to follow-up non-concordant genotoxicity results for cosmetic ingredients, we have developed, optimised and validated genotoxicity assays utilising *in vivo*-like 3D human skin models. These models replicate the physical and metabolic properties of skin and allow for safety evaluation of the dermal exposure route. In other words, these models better replicate actual human tissue and real-life exposure than traditional assays.

Thus far, results indicate good reproducibility as well as a high predictive capacity of these assays. Specifically, they show improved specificity when compared to standard *in vitro* tests, while sensitivity remains high. In 2014, the SCCS indicated that these 3D skin-based genotoxicity assays are fit for purpose by adding them to their Notes of Guidance.

NEXT STEPS

The focus in 2017 regarding the 3D-skin based genotoxicity assays will be on outreach and getting our validation data vetted by a larger audience. This will include an international community of experts from academia, regulatory bodies, and other industry partners. Further recognition will hopefully help drive wider use and acceptance of our 3D skin based genotoxicity tests.

The first outreach opportunity was a workshop in Brussels at the end of April, which brought together our task force along with recognised genotoxicity experts, biostatisticians and regulators. This will be followed by a key event in November: The International Workshop of Genotoxicity Testing (IWGT) in Tokyo, which will provide us with the opportunity to share our data with a global community of experts. The aim of the IWGT workshop is to obtain a consensus position on validation status and use of these assays.

While I am proud of the results we have achieved so far, our work remains ongoing. I look forward to updating you all on the results of our upcoming outreach activities. To mirror John's remarks: In the spirit of collaboration and dialogue, if you have questions or comments, please feel free to get in touch!

THREE QUESTIONS FOR THE GENOTOXICITY EXPERT

Dr. Rodger Curren
CEO at Institute for *In Vitro* Sciences, Inc

Q1: In your view, over the past five years, what were the needs and the related challenges for developing new, or refined, *in vitro* genotoxicity tests, and to which extent this was important for cosmetic products?

Over the past 5 years there has been a growing need to develop new, or refined, *in vitro* genotoxicity tests because of growing concerns about the high "false positive" rate of the traditional first tier battery of bacterial mutagenicity, mammalian cell mutagenicity, and/or mammalian cell cytogenetic assay. In the past a positive response in any of these *in vitro* assays could be overturned by showing negative results in an *in vivo* (animal) experiment. However recently there has been hesitancy in many industries to use the *in vivo* procedures because of consumer concerns about animal testing or company policies that restrict animal testing. The use of animal tests has become even more of a problem for the cosmetics industry because of the European Union's ban on the marketing of cosmetics products which were tested, or whose ingredients were tested, in animals.

It became a very important scientific question for cosmetic manufacturers to determine, within the constraints of legal requirements and ethical considerations, whether a potential cosmetic ingredient would be mutagenic or carcinogenic to humans. I believe the Cosmetic Europe Genotoxicity Task Force has done an excellent job in conducting programs to both improve existing assays, and to develop new ones, that will provide the

scientific information needed to more confidently determine the safety of new products and ingredients.

Q2: Regarding the development of 3D-Comet and 3D-Micronucleus, on which the task force genotoxicity was working, what were your main advices and recommendations?

My advice was the same that I would give to individuals conducting validation activities for any alternative toxicology assay. It is important: 1) to understand –and clearly express - the goal(s) of the program, 2) to be fully transparent in how the data are collected and subsequently interpreted, and 3) to involve - and work closely with - a recognized validation authority or regulatory body. I believe that that all these recommendations were followed – and continue to be followed – by the genotoxicity task force.

Q3: Still on these 3D-tests, how do you see their potential as follow-up tests to the current battery used for cosmetics?

While some additional work may be necessary, it seems clear that a rational approach is available to use 3D human tissue constructs as a follow-up to the traditional *in vitro* genotox battery. Analysis of the data developed so far indicates that materials known to have genotoxic activity when tested on animals (historic data) are also detected as positive by either the 3D-Micronucleus assay, or the 3D-Comet assay. At the same time materials known to be negative in animals have mostly been shown to be negative in both of the 3D skin models. Thus the 3D skin assays provide us with a way forward to understand the real genotoxic potential of materials that were positive in a first tier bacterial mutagenicity assay or cytogenetic assay.



The newsletter is published twice a year by Cosmetics Europe to bring you the latest news regarding the development of Alternative Approaches to Animal Testing. Each edition will focus on a particular topic of interest to the Long Range Science Strategy (LRSS) programme (2016-2020).

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