Joint Cosmetics Europe and EU-ToxRisk Virtual Workshop

3 December 2021 (1pm-5:15pm CET)

NAMs implementation into risk assessment: possibilities and challenges

Background

Cosmetics Europe (CosEu) Long Range Science Strategy programme and the EU-ToxRisk project have been running case studies based on the application of new approach methods (NAMs) in the assessment of systemic toxicity after chemical exposure. NAMs can help to strengthen the identification of suitable substances for Read-Across (RAX), increasing confidence in the analogues' identification and their suitability assessment. For RAX and ab initio evaluations, NAMs can identify the no observed adverse effect level (NOAEL) used as a point of departure (PoD) in a Next Generation Risk Assessment (NGRA). NAMs have also been used to inform on the relative potency of a mode of action (MoA) and to predict internal exposure in both human and animal studies, allowing for a risk assessment approach based on internal exposures without need to account for inter-species kinetic variability.

There are various levels of readiness of NAMs (Table 1), with several already used in the safety of cosmetics e.g., RAX and exposure-based waiving, while others e.g., organ-on-chip, are considered useful but not sufficiently developed for current use. This workshop will focus on NAMs from all three levels of readiness for which new data are being generated to support their use. These include, PBPK modelling, pharmacology profiling, cells stress panel, and organ-on chip methods. The use of transcriptomics in safety assessment has been addressed in a LRSS Workshop in March 2021 and a workshop report is available.

Table 1. Summary of new approach methodologies and their proposed state of readiness for used in the risk assessment of cosmetics ingredients (adapted from: International Cooperation on Cosmetics Regulation (ICCR). Integrated Strategies for Safety Assessment of Cosmetic Ingredients - Part 2)

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 Exposure based waiving In silico tools Metabolism and metabolite identification PBPK modelling In chemico assays Reporter gene assays 3D culture systems (local effects and genetic toxicity) Human studies	'Omics (especially transcriptomics) In vitro pharmacological profiling Pathways modelling 3D culture systems (for systemic effects)	Organ on chip

Aims of the workshop:

- **Provide a short overview of available NAMs for measuring bioactivity, exposure and hazard, and the associated levels of confidence in the conclusions derived from them.**
- **Focus on more recently developed or improved NAMs to show the advances made.**
- **To demonstrate the use NAMs in the risk assessment of cosmetics ingredients and how this approach could be translated to other regulatory frameworks.**

Agenda

13.00 – 13.05 **Welcome and housekeeping**
Mustafa Varçin (Cosmetics Europe) and Giorgia Pallocca (EU-ToxRisk)

13.05 – 13.25 **Setting the scene – Andrew White (Unilever)**
This presentation will give an overview of the different types of NAMs used to assess bioactivity and exposure and how they are used in a NGRA. Current practices will be outlined, as well as the efforts by the LRSS, NSP and EU-ToxRisk programs to evaluate NAMs for regulatory use.

13.25 – 13.55 **Use of PBPK modelling in exposure simulations: How do we qualify a model in the absence of animal data? Corie Ellison (Procter & Gamble)**
The use of PBPK modelling has universal use in different industries (especially the pharmaceutical industry) and guidelines on their use and standard are available. However, for the cosmetics industry, the challenge is to gain scientific and regulatory confidence of this NAM in the absence of *in vivo* data. This presentation will outline the requirements for a robust PBPK model and addresses how they can be qualified.

13.55 – 14.25 **NAM toolbox to cover biokinetics - Frederic Bois (CERTARA)**
In vitro toxicity testing routinely uses nominal treatment concentrations as the driver for measured toxicity endpoints. Models predicting the freely dissolved concentrations by accounting for chemical- in vitro environment interactions have been developed to address this issue. The presented model considers permeability of ionised and unionised species and accounts for membrane potential in the partitioning of ionised moieties. By accounting for lipid and protein binding in culture medium, binding to cell culture plastic, air-partitioning, and lipid binding in the cell, the model can predict chemical concentrations (free and total) in medium and cells. The model can improve in vitro in vivo extrapolation of toxicity endpoint by determining intracellular concentrations for translation to in vivo.

14.25 – 14.55 **Use of the ToxProfiler reporter assay as NAM for chemical hazard identification - Bas ter Braak (Toxys)**
A large panel of human fluorescent reporter cell lines was developed and validated at Leiden University during the EU-ToxRisk project. A critical selection of reporter cell lines that cover a wide range of toxicological

endpoints have now been combined into a single assay. This ToxProfiler assay is now offered by the Dutch CRO Toxys. ToxProfiler combines live cell confocal imaging with automated image segmentation pipelines to accurately quantify the fluorescent biomarker expression with a single cell resolution. This high throughput assay can be applied in the early chemical hazard identification field to unravel the toxicological mode of action (MoA) of chemicals.

14.55 – 15.25 **Capturing off-target effects using pharmacology profiling – progress in current targets – Gerry Kenna (Cosmetics Europe Pharmacology Profiling Working group)**

The question of how to prevent off-target toxicity was posed in a Cosmetics Europe LRSS Workshop on safety pharmacology screening for cosmetic relevant chemicals, which took place 21-22 November 2020. This "Secondary pharmacology" NAM could open additional ways to create trust in the NGRA approach of safety evaluation. As a result of the workshop, the Cosmetics Europe LRSS Systemic Toxicity Task Force is conducting a feasibility study optimize a screening panel using in vitro binding and enzymatic assays to identify and predict potential bioactivity of cosmetic-relevant chemicals. This approach is based on the knowledge that various targets of pharmacological interest have been linked to human adverse drug reactions, and the screening of these has helped the pharma industry in identifying drug candidates, as well as off-target and potential adverse effects.

15.25 – 15.55 **Use of Microphysiological (MPS) models in decision-making and safety assessment in the pharmaceutical and cosmetics industries**
Pelin Candarlioğlu (GSK) and Jochen Kühnl (Beiersdorf)

The use of MPS models has gained traction in recent years to help with the risk assessment of drugs and chemicals, and are considered higher tier and more complex models to evaluate the interaction between organs/tissues. This presentation will start with feedback from the pharmaceutical industry on the type of MPS models used to evaluate drugs and the decisions made accordingly. The second part of the presentation will describe the LRSS project using 2- and 3-organoid MPS models for evaluating the effects of the exposure route and dosing frequency on the metabolic fate of cosmetic compounds.

15.55 – 16.10 **Break**

16.10 – 17.00 **Panel discussion – Moderated by Gladys Ouédraogo (L'Oréal)**

- Which NAMs would you use in an NGRA and how much confidence do you have in the claims and conclusions of each?
- Which are the roadblocks for the mature NAMs to be implemented in regulatory dossiers?
- Where are the uncertainties and lack of confidence?
- What needs to be done to achieve sufficient confidence in NAMS that require improvement?

17.00 – 17.15 **Wrapping up and take-aways**

Cosmetics Europe Competition Law Compliance Programme

By attending the Cosmetics Europe meetings, the Cosmetics Europe Delegates acknowledge that the strict observance and compliance with competition law regulations belongs to the essential features of Cosmetics Europe's activity.

The Cosmetics Europe Delegates are aware of Cosmetics Europe's Competition Law Compliance Programme and Cosmetics Europe assumes that the Cosmetics Europe Delegates are further briefed by their own organisation as to their proper behaviour within an industry association. In case of doubt about the compliance of some discussions or activity, the Cosmetics Europe Delegates commit to revert to the Cosmetics Europe Legal Department.

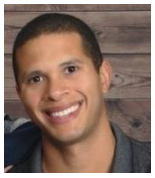
Cosmetics Europe will not enter into any discussion, activity or conduct that may infringe, on its part or on the part of its members, any applicable competition law. By way of example, the Cosmetics Europe Delegates shall not discuss, communicate or exchange any commercially sensitive information, including non-public information relating to prices, marketing and advertising strategy, costs and revenues, trading terms and conditions with third parties, including purchasing strategy, terms of supply, trade programmes or distribution strategy. This applies not only to discussions in formal meetings but also to informal discussions before, during and after meetings.

Meet the speakers

Dr. Mustafa Varçin is of Head of Science & Research at Cosmetics Europe. He has a background in Pharmaceutical (MPharm) and Cosmetic Sciences (AS) with a Ph.D. in Pharmaceutical Sciences from the Vrije Universiteit Brussel (Belgium). He is actively involved in the current state-of-the-art Long Range Science Strategy programme. He works with industry experts on innovative projects and different scientific initiatives to promote NGRA & NAMs for safety assessment of cosmetic ingredients and facilitate their regulatory use. Together with his team & industry experts, he is preparing a new global five-year science programme focusing on human health & environmental safety that will be launched soon. He joined Cosmetics Europe in September 2020.

Dr. Giorgia Pallocca is a project manager at CAAT-Europe (Center for Alternatives to Animal Testing in Europe). She has experience establishing and validating NAMs, transcriptome data analysis, test battery assembly, and scientific consortium coordination. Giorgia Pallocca obtained her MSs in 2011 in Molecular Biotechnology at the University of Bologna (Italy). Afterward, she completed a traineeship at the European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) at JRC (Italy). She obtained her Ph.D. in Toxicology in 2017 at the University of Konstanz (Germany). Since 2018, she has coordinated communication and dissemination activities within research consortia, as EU-ToxRisk and RISK-HUNT3R projects (EC-H2020).

Dr. **Andrew White** is currently a science leader in computational toxicology within the Safety and Environment Assurance Centre at Unilever. Since 2008, the focus of his work has been in the application of in vitro and in silico approaches for human risk assessment of Cosmetic ingredients, on which he has published numerous articles. Alongside this, he is actively involved in enabling modern compute capability to address this societal challenge. He continues to represent Unilever across a range of industry & scientific consortia related to the development of alternative methods. He is a member of the H2020 EU-ToxRisk and RISK-HUNT3R Steering Committee.



Dr. Corie Ellison received a Ph.D. degree in Pharmacology and Toxicology from the School of Medicine and Biomedical Sciences at The State University of New York at Buffalo. After graduation, he joined The Procter & Gamble Company as a Toxicologist in the Global Product Stewardship organization. Currently, Dr. Ellison is a Principal Scientist in the Central Product Safety division where he leads several research projects and the human safety program for multiple technologies in P&G's global cosmetic businesses. He has expertise in pharmacokinetics, i.e., Absorption, Distribution, Metabolism and Excretion (ADME), and uses this knowledge to develop innovative approaches using physiologically-based pharmacokinetic (PBPK) models to predict systemic exposure and refine/advance the quantitative risk assessment for consumer products.

Prof. Frédéric Y. Bois is an internationally known expert in pharmacokinetics, toxicology, statistics, and risk assessment. He is currently Senior Scientific Advisor and Head of Mechanistic Modelling at CERTARA UK, Simcyp Division, where he also manages external grants and the development of the Simcyp biosimulation software. When at the University of California at Berkeley, the Lawrence Berkeley Laboratory, or in France, Prof. Bois has directed research projects for the Food and Drug Administration, the National Institute of Health, the Environmental Protection Agency, the Occupational Safety and Health Administration, and the European Commission. He leads the work package on "Computational hazard estimates" in the European Commission-funded EU-ToxRisk project. He is involved at various levels in the follow-up project RISK-HUNT3R, which aims to advance the regulatory use of in vitro and computational methods for safety assessment.

Dr. Bas Ter Braak holds a Master's degree in Biotechnology from Wageningen University. After completing his Ph.D. at the Leiden Academic Centre for Drug Research (LACDR), he was a post-doctoral researcher at Leiden University. Together with a small team of Ph.D. students, he generated CRISPR/Cas9 mediated fluorophore knock-ins in human induced pluripotent stem cells to study compound-induced stress signalling processes in differentiated reporter cell lineages. He also co-developed the HepG2 BAC-GFP reporter platform. Bas joined Toxys in 2020 as a senior scientist to commercialize the HepG2 BAC-GFP reporter platform. Since 2021 he has been the project director and responsible for the ToxProfiler technology.

Dr. Gerry Kenna is a toxicologist who provides scientific advice and guidance to Cosmetics Europe's Long Range Science Strategy. In addition, he provides consultancy advice to the pharmaceutical industry on drug safety assessment and drug development, is Pharmaceutical Director of Safer Medicines Trust, (www.safermedicines.org), and is Vice President of the Board of Trustees of the Evidence-Based Toxicology Collaboration (www.ebtox.org). His work is focused on developing and implementing novel, human biology-based methods that can improve human safety evaluation of cosmetics, pharmaceuticals, and other chemicals. Before his current activities, he held toxicology leadership roles in the pharmaceutical and agrochemical industries for 15 years, having previously led academic research teams that explored mechanisms underlying serious human adverse drug reactions for 19 years. Dr. Kenna received B.Sc. and Ph.D. degrees in biochemistry from the Universities of Leeds and London. He has authored or co-authored >100 peer-reviewed scientific publications, is a Fellow of the British Toxicology Society, and is a member of the Society of Toxicology, the Drug Metabolism Discussion Group, and the International Society for the Study of Xenobiotics.

Dr. Pelin Candarlioglu is a tissue engineer by training, having received her Ph.D. in the field from Imperial College London but moved into oncology during her PostDoc position about circulating tumour cells at the UCL. Her introduction to Organ On Chip (OoC) was when she was leading a Cell Biology/Microfluidics lab in Cambridge at Enplas Corporation where she was developing a microfluidic chip system designed explicitly for IO applications for which the patent is pending. As part of Complex In Vitro Models (CIVM) group, she is leading a small team utilizing her expertise in microfluidics, tissue engineering, and especially OoC to lead multiple initiatives both externally and internally to expand the complex in vitro model portfolio of GSK for immuno-oncology. Dr. Candarlioglu is very active at the 3Rs initiative in GSK and supports reduction and replacement aspects. She also represents GSK globally in relevant organizations such as NA3RsC MPS Initiative, IQ-MPS, NC3R, OOACT in the UK, and as Chair of Industry Advisory board at EUROoCS.

Dr. Jochen Kühnl studied biology with a particular interest in molecular biology and parasitology at the University of Hamburg, Germany. He subsequently attained his Ph.D. in cell biology at the Institute for Molecular Cell Biology, Munster, Germany. In 2008, Dr. Kühnl joined Beiersdorf, a leading skincare company, to dedicate his work to alleviate skin diseases such as atopic dermatitis and psoriasis. In 2011, he joined Beiersdorf´s toxicology department as responsible manager for the Experimental Toxicology in Beiersdorf´s ´Front End Innovation´ function. His team attends to using in vitro alternatives to animal testing to assess toxicological endpoints such as skin sensitization and genotoxicity.
Additionally, the team is actively involved in evaluating microphysiological systems and 3D tissue models for cosmetic purposes. Dr. Kühnl is a member of Cosmetics Europe´s task force Skin Tolerance (working on skin sensitization assays). Additionally, he participates in a focus group project on "3D tissue models" for Cosmetics Europe´s task force ADME to evaluate the capacity of microphysiological systems to inform animal-free next-generation risk assessment.

Dr. Gladys Ouédraogo has over 19 years experience in developing predictive methods for toxicity. She leads research initiatives with external partners, mainly on repeated dose systemic toxicity and genotoxicity. Dr. Ouédraogo has a degree in Pharmacy (University of Padova), Ph.D. in Photobiology (Natural History Museum of Paris), has been a post-doctoral fellow at the Wellman Center for medicine/Harvard Medical School (Boston). She joined L'Oréal in 2003 and has held different positions related to developing alternatives to animal testing. She represents l'Oréal in other committees at Cosmetics Europe, HESI, OECD, AFSA (Animal-free safety assessment collaboration).

Workshop rapporteur

Dr. Nicky Hewitt has been active in the field of in vitro ADME-Tox research for ~30 years. She completed her doctorate degree at Mary's Hospital, London, where she continued her post-doctoral studies on hepatocyte cryopreservation techniques. Dr. Hewitt joined Merck KGaA in Germany in 1996, where she established hepatocyte models for toxicology and drug metabolism research. After several years working with two in vitro ADME-Tox CROs, she became an independent consultant in 2007. Dr. Hewitt has been a consultant for Cosmetics Europe since 2008, as the Scientific Coordinator for the Genotoxicity Task Force and later, as the Vice-Chair of the ADME Task Force. She is also a member of the Cosmetics Europe Long Range Science Strategy Core Group.