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Poster

Advancing the Application of New Approach Methodologies (NAMs) in Systemic Toxicity Assessment of Cosmetic Ingredients: Insights from Cosmetics Europe Long Range Science Strategy (2016-2022) Case Studies

Authors: **C. Mahony**[1], M. Baltazar[2], M. Dent[2], A. Giusti[3], N. Hewitt[3], A. Najjar[4], G. Ouedraogo[5], A. Schepky[4]

[1] Procter & Gamble, Reading, United Kingdom, [2] Unilever, Sharnbrook, United Kingdom, [3] Cosmetics Europe, Auderghem, Belgium, [4] Beiersdorf, Hamburg, Germany, [5] L'Oreal, Aulnay Sous-Bois, France,

1. INTRODUCTION

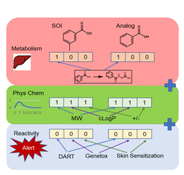
Replacing animals in toxicological safety assessment requires a fundamental change from the current animal adverse outcome driven approach to a hypothesis, mode of action driven framework. Under the Cosmetics Europe Long Range Science Strategy (2016-2022) we performed a series of 15 read across and ab initio Next Generation Case Studies to further the application of new approach methodologies (NAMs) in addressing specific hypotheses for systemic toxicity assessment, inspired by the Seurat-1 framework and the NGRA principles [1-2]. Read across case studies focused on building confidence in the use of different NAM approaches for the selection of appropriate analogues with chemistry, toxicokinetic and Mode of Action/bioactivity insights [3]. Ab initio and read across case studies rely on identifying levels of internal exposure and bioactivity [4]. For a subset of ab initio case studies, further testing was necessary based on hypotheses identified or due to uncertainties in the assessment. These included follow-ups with developmental toxicity and renal toxicity assays. In these cases, points of departure (PoDs) were compared with exposure metrics to support a safety evaluation that was at least as protective as traditional approaches.

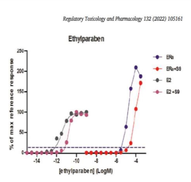
The key insights have paved the way for various projects under the International Collaboration for Cosmetic Safety (ICCS), established in 2023. This global organization aims to foster confidence in NAMs, leading to international consensus that animals are no longer necessary for the safety assessment or registration of cosmetic ingredients.

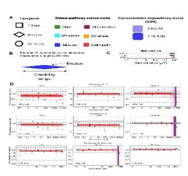
2. METHODS USED FOR CASE STUDIES (NOT AN EXHAUSTIVE LIST OF NAM)

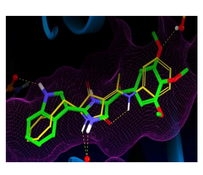
* Figures were adapted from several sources.

TO CHARACTERISE ADVERSITY/BIOLOGICAL EFFECTS *



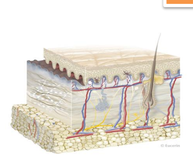


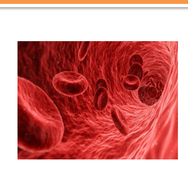


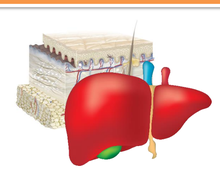


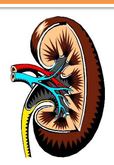
Cheminformatics	Toxicogenomics	Pharmacology Profiling *In vitro*	Functional Assays	Cell Stress Assays	*In silico Activity*
MMP & qSIM **OECD QSAR toolbox** **ChemTunes-ToxGPS** **Cosmos database** For digitized approaches to Read Across	**Transcriptomics** Various cell types (MCF-7, HepG2, HepRG, iCar, A549, PTC) for insights into MoA, functional analogues and pathways	**83 ligand affinity assays** considered in a proof of concept study	**e.g. EATS (endocr. activity)** Estrogen Androgen Thyroid Steroidogenesis	**Pathways related to cell stress and cell health** (HepG2 cells)	**e.g. Docking,** **QSAR modelling** **ER binding**

TO CHARACTERISE ADME PROPERTIES *



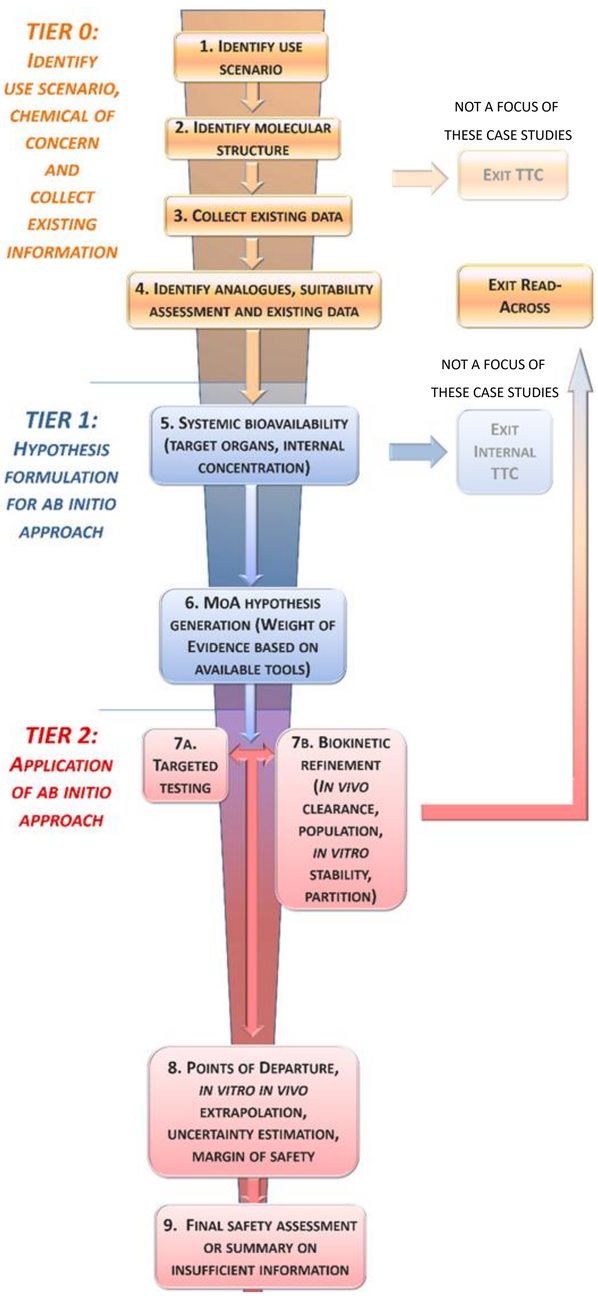






Absorption	*Distribution*	*Metabolism*	*Excretion*	*Pharmacokinetics*
Skin penetration study • *ex vivo* pig skin • *ex vivo* human skin **Gut absorption** • Caco-2	**Plasma stability** **Plasma protein binding** **Blood plasma ratio**	**Metabolic stability** • Human skin S9 mix • NHEK (Normal Human Epidermal Keratinocytes) • Primary human hepatocytes, • Plasma, e.g. esters • Multi-Organ-Chip (skin, liver)	**Renal clearance** • kidney cell monolayer • transfected cells containing human renal transporters	• **PBPK modelling** • **Clinical studies** • **Biomonitoring data**

3. CASE STUDIES



NGRA Framework, adapted from SCCS NoG [5]

READ ACROSS	• Propyl paraben and short chain parabens • Genistein and daidzein • Avobenzone • Caffeine and methylxanthines • Homosalate • 2-Ethylehexyl salicylate • Benzoic acid, salts and small alkyl derivatives	
AB INITIO	• Phenoxyethanol • Climbazole • Benzophenone 3 • Benzophenone 4 • Octocrylene • BHT • Benzyl salicylate • Octyl methoxycinnamate	
HYPOTHESIS	**METHODS**	**KEY INSIGHTS**
ANALOGUES ARE SUITABLY SIMILAR TO TARGET	• Cheminformatics and in silico (various) tools	• Critical evaluation of various analogue identification and evaluation approaches is needed • Expert judgement in read across needs to be minimised • Metabolism and functional sub-structures are important in defining categories
INTERNAL EXPOSURES CAN BE DERIVED WITH CONFIDENCE	• In vitro ADME: skin penetration, fup, blood:plasma ratio, hepatic clearance, renal clearance incl. transporters • PBPK modelling • Clinical data	• In absence of measured data, approaches to increase certainty in PBPK model predictions and underlying in vitro ADME data are needed
MOA CAN BE RELIABLY IDENTIFIED	• Toxcast data • Transcriptional profiling (different combinations of cell types) incl. CMap • Pharmacology Profiling incl. EATS • Cell stress • ReproTracker	• Use of analogues can aid identification of MoA and assessment of potency • Uncertainty exists with regards to biological coverage, i.e. systemic toxicity targets in toolbox? • Metabolic capability of in vitro systems can be improved • MoA interpretation from transcriptomics is not well harmonised
BIOKINETICS CAN BE REFINED	• Parallel Artificial Membrane Permeability Assay (PAMPA) • Transporter transfected kidney cell lines • Metabolite ID using primary human hepatocytes	• Confidence in *in vitro* parameters translates to high confidence in the simulated exposure results • Future evaluation of PBPK performance and uncertainty quantification is needed for chemical substrates of transporters
IN VITRO POINTS OF DEPARTURE ARE PROTECTIVE FOR *IN VIVO* EFFECTS	• Toxcast data • Transcriptional profiling • Pharmacology Profiling • Specific organ toxicity e.g. proximal tubule cells	• In vitro PoDs were protective for the chemistries under investigation • Uncertainty exists with regards bioactivity i.e. versus adversity • Low toxicity hypotheses can be aided by understanding differences in activity of chemicals within a broader category and comparison with *in vivo* exposures

4. CONCLUSIONS AND FURTHER WORK

- The collective experience, along with the various hypotheses explored and methodology used in the different cases, has led to increased confidence in an exposure-led, mode of action driven framework for systemic toxicity assessment
- Read across allows the use of analogues to fill data gaps and aid in supporting mode of action and potency assessments
- A combination of non-targeted broad testing approaches combined with targeted assays provided measures of bioactivity which appear to be at least as protective as traditional approaches. Conservatism in the approach could be refined by employing higher tier testing to distinguish bioactivity from adversity, and to test the biological and chemical coverage of the NAMs.
- Key insights have been translated (or are currently being translated) into projects under ICCS Human Health Science - Advancing Animal-Free Science for Cosmetics (iccs-cosmetics.org)
- Finally, opportunities are sought to evaluate new approaches in a NGRA context, to understand the strengths and limitations. The placement of the LRSS case study chemicals within cosmetic, food additive, REACH and CLP regulation offers occasion to do so across industrial sectors

5. REFERENCES

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3 - Alexander-White, C. et al. (2022). A 10-step framework for use of read-across (RAX) in next generation risk assessment (NGRA) for cosmetics safety assessment. Regul. Toxicol. Pharmacol. 129, 105094. doi:10.1016/j.yrtph.2021.105094.

4 - Berggren, E. et al. (2017). Ab initio chemical safety assessment: a workflow based on exposure considerations and non-animal methods. Comput. Toxicol. 4, 31–44. doi:10.1016/j.comtox.2017.10.001

5 - SCCS (2021). Scientific committee on consumer safety, SCCS notes of guidance for the testing of cosmetic ingredients and their safety evaluation 11th revision. SCCS/1628/21

Check out LRSS publications