A CASE STUDY APPROACH TO NEXT GENERATION RISK ASSESSMENT FOR CONSUMER SAFETY OF COSMETICS

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CHANGING GLOBAL ENVIRONMENT

- Animal testing of cosmetic products and ingredients (and sale of) prohibited throughout the European Union since 11\textsuperscript{th} March, 2013
- Several countries across globe followed suit, India since June 2013
- 2007-2018: More than a decade of inspiration to application to Next Generation RISK Assessments (NGRA)

“Advances in toxicogenomics, bioinformatics, systems biology, epigenetics, and computational toxicology could transform toxicity testing from a system based on whole-animal testing to one founded primarily on \textit{in vitro} methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin.” 2007

“A primary objective for improving exposure science is to build confidence in the exposure estimates used to support risk-based decision-making, by enhancing quality, expanding coverage and reducing uncertainty ……An important focus has been on the development of PBPK models for translating exposures between test systems and human exposure scenarios” 2018
SUCCESES IN ANIMAL ALTERNATIVES

• Internationally accepted toxicity tests that do not use animals
• Guidelines published by OECD
Can we use a new ingredient safely?

Can we safely use $x\%$ of ingredient $y$ in product $z$?
MAXIMISING USE OF EXISTING INFORMATION AND NON-ANIMAL APPROACHES

- All available safety data
- in silico predictions
- Exposure-based waiving approaches
- History of safe use
- Read across
- Use of existing OECD in vitro approaches
Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients

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ICCR NINE PRINCIPLES OF NEXT GENERATION RISK ASSESSMENT (NGRA)

4 Main overriding principles:
• The overall goal is a human safety risk assessment
• The assessment is exposure led
• The assessment is hypothesis driven
• The assessment is designed to prevent harm

3 Principles describe how a NGRA should be conducted:
• Following an appropriate appraisal of existing information
• Using a tiered and iterative approach
• Using robust and relevant methods and strategies

2 Principles for documenting NGRA:
• Sources of uncertainty should be characterized and documented
• The logic of the approach should be transparently documented
A CASE STUDY APPROACH – IMAGINE WE HAD NO DATA ...
EXPOSURE-LED

Exposure scenario:
- Worst case in US: 32.97 µg/cm²
- Used one time per day
- Skin surface area: 4712.5 cm² (95 percentile)
- Amount of product used per day: 5.18 g/day
- Amount of ingredient in contact with skin: 155 mg/day

PBPK model predicted free concentrations (µM)

<table>
<thead>
<tr>
<th></th>
<th>plasma</th>
<th>heart</th>
<th>liver</th>
<th>brain</th>
<th>adipose</th>
</tr>
</thead>
<tbody>
<tr>
<td>C0-41.7</td>
<td>3.1</td>
<td>0.6-7.2</td>
<td>0.2</td>
<td>0.01</td>
<td>0.002</td>
</tr>
<tr>
<td>C0-0.15</td>
<td>0.015</td>
<td>0.01</td>
<td>0.01</td>
<td>0.003</td>
<td>0.0003</td>
</tr>
<tr>
<td>C0-0.03</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
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<td>0.003</td>
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</tbody>
</table>

Skin penetration

Caffeine Free Concentration

Laundry scenarios

<table>
<thead>
<tr>
<th>Exposure scenario</th>
<th>Systemic exposure (mg/kg bw per day)</th>
<th>Local (µg/cm²)</th>
<th>Total exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro-Treatment</td>
<td>0.19</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Main wash</td>
<td>0.3</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td>Residues on clothes</td>
<td>0.06</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Total (Main wash + residues on clothes)</td>
<td>0.36</td>
<td>8.44</td>
<td></td>
</tr>
<tr>
<td>Total (post-treatment + main wash + residues on clothes)</td>
<td>0.55</td>
<td>58.44</td>
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</tbody>
</table>

Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Model 4 output</th>
<th>Model 4 output</th>
<th>Model 5 output</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (µg/mL)</td>
<td>0.68 µM</td>
<td>0.46(1.15 µM)</td>
<td></td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>48.85</td>
<td>48.85</td>
<td></td>
</tr>
<tr>
<td>AUC0-inf (µg-h/mL)</td>
<td>1.73</td>
<td>8.64</td>
<td></td>
</tr>
<tr>
<td>AUC0-t (µg-h/mL)</td>
<td>1.74</td>
<td>8.57</td>
<td></td>
</tr>
<tr>
<td>Cmax Liver</td>
<td>0.068 µM</td>
<td>0.061(0.4 µM)</td>
<td></td>
</tr>
</tbody>
</table>
A TIERED AND ITERATIVE APPROACH

**Tier 1**

**IN SILICO-FIRST**

**EXAMPLES:**
- MIE *in silico* Atlas & QSARs
- Skin haptenation modelling
- *In silico* receptor screening

*In silico*-first approaches for identifying pathways of concern, building weight of evidence and formulating hypotheses for testing

**Tier 2**

**PATHWAY IDENTIFICATION (TARGETS AND OFF-TARGETS)**

**EXAMPLES:**
- HT-Transcriptomics
- *In vitro* screening panels
- High content imaging
- SPME free concentration

Identifying/characterising lead MIEs and pathways through experimental data generation, informatics data mining and computational modelling

**Tier 3**

**PATHWAY CHARACTERISATION (TARGETS)**

**EXAMPLES:**
- 3D and organotypic cell models
- Molecular dynamic simulations
- Integrated *in vitro* systems

Characterisation of response in biologically relevant *in vitro* systems or complex computational models for decision making

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**Using 2D Structural Alerts to Define Chemical Categories for Molecular Initiating Events**

Timothy E. H. Allen, Jonathan M. Goodman,*† Steve Gutsell,* and Paul J. Russell*†

*Centre for Molecular Informatics, Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, United Kingdom, and Unilever Safety and Environmental Assurance Centre, Guilworth Science Park, Sharnbrook, Bedfordshire MK44 1LQ, United Kingdom

* Correspondence: Timothy.E.Allen@cam.ac.uk; Tel.: +44-1223-760-835; Fax: +44-1223-761-150
The SARA Weight of Evidence (WoE) human potency model* is a high-dimensional probability distribution describing data from the following sources:

- DPRA OECD TG442D (cys/lys depletion)
- KeratinoSens™ OECD TG442C (EC1.5, EC3, IC50)
- H-Clat OECD TG442E (CD54 EC200, CD86 EC150)
- U-SENS™ OECD TG 442E (CD86)


AOP for skin sensitisation
https://aopwiki.org/aops/40
PREDICTION OF PROBABILITY OF SENSITISATION OCCURRING IN HRIPT FOR CASE STUDY CHEMICALS

- DNCB
- Methyl heptine carbonate
- Coumarin
- Lactic acid

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= No Expected Sensitization Induction Level

(www.ifraorg.org)
PROBABILITY OF CONSUMER BECOMING SENSITISED

Face cream

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Chance of inducing sensitisation in at least one individual</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNCB</td>
<td>p=1.00</td>
</tr>
<tr>
<td>Methyl heptine carbonate</td>
<td>p=0.19</td>
</tr>
<tr>
<td>Coumarin</td>
<td>p=0.00</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>p=0.00</td>
</tr>
</tbody>
</table>

Shampoo

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Chance of inducing sensitisation in at least one individual</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNCB</td>
<td>p=0.91</td>
</tr>
<tr>
<td>Methyl heptine carbonate</td>
<td>p=0.01</td>
</tr>
<tr>
<td>Coumarin</td>
<td>p=0.00</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>p=0.00</td>
</tr>
</tbody>
</table>
TOXCAST: COMBINING IN VITRO ACTIVITY AND DOSIMETRY

Range of in vitro AC50 values converted to human in vivo daily dose

Safety margin

Actual Exposure (est. max.)


Slide from Dr Rusty Thomas, EPA, with thanks
UNILEVER/US EPA 2015-2020: JOINT CRADA

Cooperative Research and Development Agreement with the United States Environmental Protection Agency

This Cooperative Research and Development Agreement (CRADA or "Agreement") is entered into by and between UNILEVER UK CENTRAL RESOURCES LIMITED (a company incorporated in England and Wales (registered under number 00029140) and whose registered office is at Unilever House, 100 Victoria Embankment, London EC4Y 0DY, UK ("the Cooperator"); and the National Center for Computational Toxicology ("the Center"), of the U.S. Environmental Protection Agency ("EPA") under the authority of Title 15, United States Code §§1710a-3710d (commonly known as the Federal Technology Transfer Act of 1986).

Case Study Chemicals

- Caffeine
- Curcumin
- Bisdemethoxycurcumin
- Tetrahydrocurcumin
- 6-Gingerol
- Coumarin
- Hydroquinone
- Doxorubicin

1. ToxCast and other technologies for i.d. of MIEs
   - Determine pathway perturbations by ingredients
2. Generate inputs to toxicokinetics
3. High throughput transcriptomics (Tempo-Seq)
4. Evaluate comp tox methods to inform risk assessments and read-across methods
5. Translation of results into NGRA
   - POD/BPAD/NOTEL/RD/IVIVE
6. Decision making despite uncertainty
7. Assess health risks of chemical ingredients without animal studies
8. Better reflect the actual risk associated with intended human exposure
   - Not rodent apical end-points
CELL STRESS PANEL

14 chemicals, including

- 'Low-risk' compounds:
  - Phenoxyethanol
  - Niacinamide
  - Caffeine

- Known 'high-risk' compounds:
  - Doxorubicin
  - Diclofenac
  - Troglitazone

Platforms

- Technology: High content imaging
- Cell line: HepG2
- Timepoints: 1, 6 & 24 hours

Calculate ‘free concentration’

**Use in vitro exposure models:**

Groothuis et al (2015) Toxicology, 332, 30-40
**CELL STRESS PANEL**

**Coumarin**

6 hours

- Cellular ATP 6h
- IL-6 6h
- XBP1 6h
- LDH release 6h
- Intracellular pH 6h

24 hours

- Glutathione content 24h
- Cellular ATP 24h
- IL-8 24h
- ICAM-1 24h
- ICAM1 24h
- HIF alpha 24h
- Phospholipidosis 24h

**Doxorubicin**

6 hours

- SRXN1 6h
- Glutathione content 6h
- AHR Translocation 6h
- Oxidative stress 6h
- Mitochondrial mass 6h
- Cellular ATP 6h
- Metastation 6h
- Heat Shock Response (Hsp70) 6h
- HIF-alpha 6h
- ATF4 6h
- DNA damage (p-H2AX) 6h
- DNA structure 6h
- Caspase 3/7 intensity 6h

24 hours

- Oxidative stress 24h
- Glutathione content 24h
- AHR Translocation 24h
- Mitochondrial mass 24h
- PGC1alpha 24h
- Mitochondrial ROS 24h
- Cellular ATP 24h
- NRF1 24h
- NRF2 24h
- IL-8 24h
- HIF1alpha 24h
- Endoplasmic Reticulum 24h
- PERK 24h
- ATF4 24h
- Phospho-p38 24h
- DNA damage (p-H2AX) 24h
- DNA repair 24h
- Cell cycle arrest 24h
- Nuclear size 24h
- DNA structure 24h
- LDH release 24h
- Caspase 3/7 intensity 24h

Quantify evidence of a response, calculate PoD
HIGH THROUGHPUT TRANSCRIPTOMICS

NOTEL* is the derived concentration of a compound that does not elicit a meaningful change in gene expression (i.e. the threshold of the concentration that elicits minimal mechanistic activity).

Recommended approaches in the application of toxicogenomics to derive points of departure for chemical risk assessment
Farmahin et al (2017) Arch Tox 91, 2045-65

*NOTEL = No observed transcriptional effect level

ICCR PRINCIPLES OF RISK ASSESSMENT AND WHAT WE’RE LEARNING FROM CASE STUDIES

• Importance of understanding consumer exposure including the relevance of metabolism
• Non-standard, bespoke data generation driven by the risk assessment question
• Ensuring quality, robustness of non-standard (non-TG, non-GLP?) work. In silico modelling approaches and bespoke in vitro solutions
• Importance of defining points-of-departure and understanding adverse vs. adaptive responses
• Understanding uncertainty in risk assessments to allow informed decision-making
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Unilever
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