Next Generation Risk Assessment (NGRA): A case study approach

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Next Generation Risk Assessment (NGRA)
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

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

Principles for documenting NGRA:
- Sources of uncertainty should be characterized and documented
- The logic of the approach should be transparently and documented
Case Study Approach... Imagine we have no data for: **Coumarin**

Safety assessment required for 0.1% coumarin in Face Cream

Safety assessment required for 0.1% coumarin in Body lotion

Baltazar et al (2020) Toxicological Sciences, Accepted
In vitro Bioactivity Characterisation

Determine Margin of Safety

Risk Assessment Conclusion

**TIER 0**

- Exposure Estimation
- Collate Existing Information
  - Local and systemic exposure estimates
    - Use scenario
    - Consumer Habits
    - Applied Dose
    - ADME parameters
    - Exposure (PBK)

- Problem Formulation
  - Molecular Structure
  - *In silico* predictions
  - Literature

**TIER 1**

- Initial PoD identification
  - ToxTracker
  - SafetyScreen44
  - BioMap® Diversity 8 Panel
  - Cell Stress Panel
  - HTTr – TempO-Seq

- Concentration-Response analysis
  - PoD* in vitro

- In vitro Refinement
  - Increased certainty in PoD and IVIVE
    - Metabolite identification
    - *In vitro* kinetics
    - 3D Models

- Plasma C\textsubscript{max}

**TIER 2**

- Insufficient data and/or low certainty
- Sufficient data and high certainty

- High risk or Low risk conclusion based on the margin of safety calculations.
Physiologically-based kinetic modelling using GastroPlus® v9.5.

Estimations based on experimental data (Clint, fup, bpr, solubility, LogP). Skin penetration parameters were fitted against skin penetration data.

### Key output parameters from uncertainty analysis:

<table>
<thead>
<tr>
<th></th>
<th>Total Plasma</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µM)</th>
<th>Mean</th>
<th>Median</th>
<th>90th percentile</th>
<th>95th percentile</th>
<th>97.5th percentile</th>
<th>99th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Face Cream</strong></td>
<td></td>
<td></td>
<td>0.0022</td>
<td>0.0021</td>
<td>0.004</td>
<td>0.0043</td>
<td>0.0046</td>
<td>0.005</td>
</tr>
<tr>
<td><strong>Body Lotion</strong></td>
<td></td>
<td>0.01</td>
<td>0.01</td>
<td>0.018</td>
<td>0.019</td>
<td>0.02</td>
<td>0.022</td>
<td></td>
</tr>
</tbody>
</table>

**Uncertainty & Population Variability**

**Physiologically-based kinetic modelling using GastroPlus® v9.5.**

Moxon et al (2020) Toxicology in Vitro, 63 104746
**Ab initio NGRA Framework**

**Tier 0**
- Exposure Estimation
  - Use scenario
  - Consumer Habits
  - Applied Dose
  - ADME parameters
  - Exposure (PBK)
- Collate Existing Information
  - Molecular Structure
  - In silico predictions
  - Literature

**Tier 1**
- **In vitro Bioactivity Characterisation**
  - Initial PoD identification
    - ToxTracker
    - SafetyScreen44
    - BioMap® Diversity 8 Panel
    - Cell Stress Panel
    - HTTr - Temp0-Seq
  - Determine Margin of Safety
    - Concentration-Response analysis
- **In vitro Refinement**
  - Increased certainty in PoD and IVIVE
    - Metabolite identification
    - In vitro kinetics
    - 3D Models

**Tier 2**
- Risk Assessment Conclusion
  - Plasma C_{\text{max}}
  - Sufficient data and high certainty
  - Insufficient data and/or low certainty
  - High risk or Low risk conclusion based on the margin of safety calculations.
All binding and enzymatic assay results were negative at 10 μM, including COX-1 and COX-2.

No receptor/target-led pharmacological effect.
**Immunomodulatory Bioactivity: BioMap® Diversity 8 Panel**

BioMAP systems contain human primary cell types (or combinations) that are stimulated to replicate complex cell and pathway interactions of vascular inflammation, immune activation and tissue remodelling.

**Biological readouts associated with anti-proliferative and tissue remodelling activities across all cell systems**

No immunomodulatory effects at relevant concentrations

Data suggest that coumarin is not an anti-inflammatory compound

*Biocorrelation is significantly changed outside of the vehicle envelops, occurs at 2 or more consecutive concentrations, and the % change is >20 for at least one concentration

*Biocorrelation is significantly changed outside of the vehicle envelops, a dose response is seen, however, the % change is <20 at the top dose
In Vitro Bioactivity: Cell Stress Panel

- 40 Biomarkers; 3 Timepoints; 8 Concentrations; ~10 Stress Pathways

**Step 1**
Selection of stress pathways
- Mitochondrial Toxicity, Oxidative Stress, DNA damage, Inflammation, ER Stress, Metal Stress, Heat Shock, Hypoxia, Cell Health

**Step 2**
Selection of chemicals according to different classes and exposure scenarios (based on typical use of compound)
- Non-stress inducers
  - Caffeine (beverages, cosmetics)
  - Coumarin (food, cosmetics)
  - Niacinamide (food, cosmetics)
  - Phenoxethanol (cosmetics)
- Stress inducers
  - CDDO-Me (drug)
  - Sulforaphane (food)
  - DEM (industrial chemical)
  - tBHQ (antioxidant)
  - Doxorubicin (drug)
  - Diclofenac (drug)
  - Triclosan (antimicrobial)
  - Trogitzone (drug)
  - Potglitzone (drug)
  - Rositzone (drug)

**Step 3**
Selection of in vitro concentrations based upon realistic human exposures
- Information on human exposure obtained from human clinical trials or PBK modelling
- Selection of 8 in vitro concentrations (upper bound limited by ~20% cytotoxicity)

Key
- Exposure scenario adopted for chemical is high risk (from consumer goods perspective).
- Exposure scenario adopted for chemical is low risk (from consumer goods perspective).

Hatherell et al (2020) Toxicological Sciences, Accepted
**In Vitro Bioactivity: Cell Stress Panel**

### Summary with PoD for cell stress biomarkers:

- **Coumarin** not very active in comparison to known ‘high risk compounds’ like doxorubicin, diclofenac etc.

- Cell count, cellular ATP, GSH, IL-8, Phospholipids, OCR, reserve capacity and steatosis showed a dose response.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Cell type</th>
<th>Stress pathway</th>
<th>PoD (µM)</th>
<th>Effect</th>
<th>Concentration dependency score (CDS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP (6h)</td>
<td>HepG2</td>
<td>cell health</td>
<td>794 [363-977]</td>
<td>down</td>
<td>0.98</td>
</tr>
<tr>
<td>ATP (24h)</td>
<td>HepG2</td>
<td>cell health</td>
<td>617 [282-891]</td>
<td>down</td>
<td>1</td>
</tr>
<tr>
<td>Phospholipidosis (24h)</td>
<td>HepG2</td>
<td>cell health</td>
<td>759 [437-977]</td>
<td>down</td>
<td>0.93</td>
</tr>
<tr>
<td>GSH (24h)</td>
<td>HepG2</td>
<td>oxidative stress</td>
<td>851 [301-1000]</td>
<td>up</td>
<td>0.92</td>
</tr>
<tr>
<td>IL-8 (24h)</td>
<td>HepG2</td>
<td>inflammation</td>
<td>912 [575-1000]</td>
<td>down</td>
<td>0.61</td>
</tr>
<tr>
<td>OCR (1h)</td>
<td>NHEK</td>
<td>mitochondrial toxicity</td>
<td>62 [2.6-776]</td>
<td>down</td>
<td>0.6</td>
</tr>
<tr>
<td>OCR (6h)</td>
<td>NHEK</td>
<td>mitochondrial toxicity</td>
<td>468 [214-794]</td>
<td>down</td>
<td>1</td>
</tr>
<tr>
<td>OCR (24h)</td>
<td>NHEK</td>
<td>mitochondrial toxicity</td>
<td>309 [138-1000]</td>
<td>down</td>
<td>0.52</td>
</tr>
<tr>
<td>Reserve capacity (1h)</td>
<td>NHEK</td>
<td>mitochondrial toxicity</td>
<td>44 [23-96]</td>
<td>down</td>
<td>1</td>
</tr>
<tr>
<td>Reserve capacity (6h)</td>
<td>NHEK</td>
<td>mitochondrial toxicity</td>
<td>759 [302-1000]</td>
<td>down</td>
<td>0.9</td>
</tr>
<tr>
<td>Reserve capacity (24h)</td>
<td></td>
<td></td>
<td>794 [295-1000]</td>
<td></td>
<td>0.55</td>
</tr>
</tbody>
</table>
High-Throughput Transcriptomics Gene Expression Profiling (HTTr)

**Defining a safe operating exposure for systemic toxicity using a NOTEL (No Transcriptional Effect Level)**

**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)

**Cell lines (chosen to express a range of relevant receptors)**
- MCF-7 – human breast adenocarcinoma cell line
- HepG2 – human liver carcinoma
- HepaRG – terminally differentiated hepatic cells that retain many characteristics of primary human hepatocytes + as spheroids
- N-HEK – primary normal human epidermal keratinocytes

*In Vitro Bioactivity: Tempo-Seq Technology*
**In Vitro Bioactivity: Tempo-Seq Technology**

- Coumarin dose range 0.001uM to 100uM
- 24 hour time point
- QC and normalisation in DESeq2
- BMDExpress2 applied to determine NOTEL (3 pathway approaches)
PoDs and plasma $C_{\text{max}}$ (µM) are expressed as total concentration

$C_{\text{max}}$ expressed as a distribution:
- Line = median (50th percentile)
- Inner band = 25th-75th percentile
- Outer band = 2.5th-97.5th percentile (95th credible interval)

Margin of Safety considering PODs and Exposure

PoDs and plasma $C_{\text{max}}$ (µM) are expressed as total concentration

$C_{\text{max}}$ expressed as a distribution:
- Line = median (50th percentile)
- Inner band = 25th-75th percentile
- Outer band = 2.5th-97.5th percentile (95th credible interval)
Application of Ab Initio Approach: Risk Assessment (NGRA)

Margin of safety is the fold difference between the Cmax and the in vitro POD.
"The primary objective of this work was to compare PODs based on high-throughput predictions of bioactivity, exposure predictions, and traditional hazard information for 448 chemicals"
Conclusions

Non-animal safety assessments for cosmetics are moving from 'might be possible in theory' to 'case studies to evaluate'

NGRA is a framework of non-standard, bespoke data-generation, driven by the risk assessment questions

• Enabling a transition from using data from tests in live animals to one founded on understanding the effects of chemicals in humans using computational approaches and in vitro methods that evaluate changes in biologic processes using human cells
• Constructed from in silico modelling approaches and in vitro solutions
• Need to ensure quality/robustness of the non-standard (non-TG) work
• Importance of characterising uncertainty to allow informed decision-making
• Shortcomings will be addressed by current and future research
• More research, creativity and published examples needed to increase confidence for regulatory application.

The approaches and challenges are not cosmetic-specific, how can different sectors learn together?
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