Integration of new approach methodologies for cosmetic safety decision making

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Use of Existing OECD *In Vitro* Approaches

Skin and eye irritation; skin sensitization; phototoxicity; mutagenicity... what about systemic toxicity?
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

In Vitro Bioactivity vs Bioavailability

“Protection not Prediction”

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

Hepatic clearance and plasma protein binding determinations

Safety margin

Actual Exposure (est. max.)

Slide from Dr Rusty Thomas, EPA, with thanks

414/448 chemicals = 92% of the time this naïve approach appears conservative

Katie Paul-Friedman et al. 2019 Tox Sciences, October Issue

Efforts to Reduce Animal Testing at EPA

On September 10, 2018, EPA Administrator Andrew Wheeler signed a directive that prioritizes efforts to reduce animal testing. The memorandum calls for the agency to:

- reduce RQs and funding of mammalian studies by 30 percent by 2025, and
- eliminate all mammalian study requests and funding by 2035.
The Margin of Safety Approach

Point of Departure

Exposure models (PBK, free/total concentration)

Point of departure derived from \textit{in vitro} concentration-response

Margin of safety

$C_{\text{max}}$
Case Study Approach... Imagine we have no data for: Coumarin

Baltazar et al., (2020) *Toxicological Sciences*, accepted

Safety assessment required for 0.1% coumarin in Body Lotion

Safety assessment required for 0.1% coumarin in Face Cream
Case Study Framework

Exposure Estimation
- Local and systemic exposure estimates
  - Use scenario
  - Consumer Habits and Practices
  - Applied Dose
  - ADME parameters
  - Internal Exposure (PBPK)

Collate Existing Information
- Problem Formulation
  - Molecular Structure
  - In silico predictions
  - Literature

In Vitro Biological Activity Characterization
- Initial PoD identification
  - ToxTracker
  - SafetyScreen44®
  - BioMap® Diversity 8 Panel
  - Cell Stress Panel
  - HTTox - TempO-Seq

Metabolism refinement
- Insufficient data and high uncertainty
- Increased certainty in PoD and IVIVE
  - Metabolite identification
  - 3D Models

Determine Margin of Safety
- PoD derived
- Sufficient data and high certainty

Risk Assessment Conclusion
- Low risk conclusion based on the margin of safety calculations.

Plasma $C_{\text{max}}$
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.
All binding and enzymatic assay results were negative at 10 uM, including COX-1 and COX-2.

No receptor/target-led pharmacological effect.
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.

*Log ratio (compound/DMSO control)*

<table>
<thead>
<tr>
<th>Biomarker affected (-20%)</th>
<th>Proliferation (-23%)</th>
<th>Tissue Factor (-26%)</th>
<th>MMP-1 (-27%)</th>
<th>Proliferation (-25%)</th>
<th>Proliferation (-44%)</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DR (-13%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>Eotaxin-3 (-14%)</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>IL-1α (-14%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>18.5 µM</td>
<td>56 µM</td>
<td>167 µM</td>
<td>500 µM</td>
<td>18.5 µM</td>
<td>56 µM</td>
<td>167 µM</td>
</tr>
</tbody>
</table>

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

*Biomarker is significantly changed outside of the vehicle envelopes, a dose response is seen, however, the % change is ≤20 at the top dose
### In Vitro Bioactivity: Cell Stress Panel

Hatherell et al., (2020) Toxicological Sciences, accepted

#### 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, LDH, ICAM-1 and steatosis showed a dose response

#### Table:

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Stress pathway</th>
<th>PoD (2.5&lt;sup&gt;th&lt;/sup&gt; percentile), µM</th>
<th>PoD (50&lt;sup&gt;th&lt;/sup&gt; percentile), µM</th>
<th>PoD (97.5&lt;sup&gt;th&lt;/sup&gt; percentile), µM</th>
<th>Effect</th>
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</thead>
<tbody>
<tr>
<td>Cell count (72h)</td>
<td>Cell health</td>
<td>54</td>
<td>150</td>
<td>316</td>
<td>down</td>
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<tr>
<td>ATP (6h)</td>
<td>Cell health</td>
<td>411</td>
<td>738</td>
<td>976</td>
<td>down</td>
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<tr>
<td>ATP (24h)</td>
<td>Cell health</td>
<td>194</td>
<td>449</td>
<td>763</td>
<td>down</td>
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<tr>
<td>GSH (24h)</td>
<td>Oxidative stress</td>
<td>641</td>
<td>781</td>
<td>979</td>
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<tr>
<td>IL-8 (6h)</td>
<td>Inflammation</td>
<td>8.8</td>
<td>52</td>
<td>123</td>
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<td>IL-8 (24H)</td>
<td>Inflammation</td>
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<td>Phospholipidosis (24h)</td>
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<tr>
<td>Phospholipidosis (72h)</td>
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<td>ICAM-1 (24h)</td>
<td>Inflammation</td>
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<td>Steatosis</td>
<td>Cell health</td>
<td>59</td>
<td>659</td>
<td>974</td>
<td>up</td>
</tr>
</tbody>
</table>
**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)
Case Study Framework

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Determine Margin of Safety

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Graph: Distributions of Oral Equivalent Values and Predicted Chronic Exposures
Margin of Safety considering PODs and Exposure

PoDs and plasma $C_{\text{max}}$ ($\mu$M) 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

<table>
<thead>
<tr>
<th>Technology</th>
<th>Cell line/Enzyme/Biomarker</th>
<th>Face cream Min. 5th percentile MoS</th>
<th>Body Lotion Min. 5th percentile MoS</th>
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</thead>
<tbody>
<tr>
<td>Cell stress panel</td>
<td>HepG2 [ATP, 24h]</td>
<td>96738</td>
<td>22048</td>
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<td>Cell stress panel</td>
<td>NHEK [OCR 1h]</td>
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<td>PubChem</td>
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<tr>
<td>HTTr</td>
<td>HepaRG_3D_24h</td>
<td>9538</td>
<td>2137</td>
</tr>
</tbody>
</table>
Conclusions

Available tools can be integrated to make a safety decision

- NGRA is a framework of non-standard, bespoke data-generation, driven by the risk assessment questions
- As applied here it is protective not predictive
- Need to ensure quality/robustness of the non-standard (non-TG) work and to characterise uncertainty to allow informed decision-making
  - Rethinking MoS/MoE
- Shortcomings will be addressed by current and future research
- More research, creativity and examples needed to land this successfully across the community
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