Next Generation Systemic Toolbox

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Conflict of Interest Statement

- The research described in this session was supported by an entity that manufactures and/or distributes a material that is the subject of this session.
- Mention of specific products does not constitute an endorsement of those products.
Objectives

- To introduce one approach to non-animal safety decision making
- To explain the International Cooperation on Cosmetics Regulation
- Principles of Next Generation Risk Assessment
- To describe some of the tools that can be used and how a decision can be reached
An exposure-led, hypothesis driven risk assessment approach that incorporates one or more NAMs to ensure that chemical exposures do not cause harm to consumers

Principles of 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 describe how a NGRA should be documented:
  - Sources of uncertainty characterized and documented
  - The logic of the approach transparent and documented
“Protection Not Prediction”

Rotroff et al. 2010
EPA, NTP, HC, A*STAR, ECHA, EFSA, JRC, RVIM…

Katie Paul-Friedman et al. (2019)

414/448 chemicals = 92% of the time this naïve approach appears conservative
The Core NAMs in Our Systemic NGRA Toolbox

PBK Modelling

- Genetic Tox
- ToxTracker/Ames/In vitro
- Micronucleus

In vitro pharmacological profiling

- Moxon et al., 2020

Transcriptomics

- Use of full human gene panel - 21k
- 24 hrs exposure
- 7 concentrations
- 3 cell lines HepG2/HepaRG/MCF7
- 3D HepaRG spheroid

BMDexpress 2

- Reynolds et al., 2020

Cellular Stress Pathways

- 13 chemicals, 36 Biomarkers; 3 Timepoints; 8 Concentrations; ~10 Stress Pathways

Hatherell et al., 2020
Example NGRA: Hexylresorcinol

- HR uses include as an approved food additive in the EU
  - Prevention of melanosis in shrimp
  - Scientific Opinion on the re-evaluation of 4-hexylresorcinol (E 586) as a food additive (wiley.com)

- How would you use the NGRA toolbox instead of the animal data to assess this use?
Tiered Approach to Exposure Estimation

- **Level 0**: Characterize Exposure Scenario
  - Maximum Permitted Level in EU is 2 mg/kg shrimp
  - 95th %ile intake (consumers only) 3.3 µg/kg/day (Adults, 18-64 y)

- **Level 1**: PBK model built with *in silico* parameters only
  - Predicted plasma $C_{\text{max}} = 0.007$ µM

- **Level 2**: PBK model built with *in vitro* parameters
  - Predicted plasma $C_{\text{max}} = 0.006$ µM

- **Level 3**: PBK model improved with *in vivo* data
  - N/A: none available for HR

Moxon et al., 2020
Bioactivity Data (1/3)

Cell Stress Global Point of Departure=3.8 µM

Middleton et al. (2022)
Bioactivity Data (2/3)

- IPP dose response for
  - A: PTGS1 (COX-1), 95% C.I.(IC50) = [0.2µM, 0.4µM]
  - B: PTGS2 (COX-2), 95% C.I.(IC50) = [1.4µM, 2.1µM]
  - C: HTR2B (serotonin receptor 2B), 95% C.I.(IC50) = [5.7µM, 9.6µM]
  - D: SLC6A2 (norepinephrine transporter), 95% C.I.(IC50) = [7.3µM, 9.5µM]

Middleton et al. (2022)
Bioactivity Data 3/3

- High throughput transcriptomics data analysed using 2 methods:
  - BIFROST (Bayesian inference for region of signal threshold): Minimum effect concentration across all genes.
  - Benchmark Dose Lower Confidence Interval (BMDL_{10})

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Global PoD (µM)</th>
<th>Minimum Pathway BMDL (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HepaRG</td>
<td>8.1</td>
<td>53</td>
</tr>
<tr>
<td>HepG2</td>
<td>7.3</td>
<td>27</td>
</tr>
<tr>
<td>MCF7</td>
<td>0.8</td>
<td>15</td>
</tr>
</tbody>
</table>

Middleton et al. (2022)
Bioactivity: Exposure Ratio

- Ratio of lowest PoD and Exposure
- 2.5\textsuperscript{th}, 50\textsuperscript{th}, 97.5\textsuperscript{th} percentile BERs:
  
  \begin{tabular}{|c|c|c|}
  \hline
  2.5 & 51 & 1100 \\
  \hline
  \end{tabular}

Middleton et al. (2022)
Toolbox Evaluation (Pilot Phase)

Are NAM-based assessments protective? What BER is needed to assure safety?

Yellow: High Risk Exposure Scenarios
Blue: Low Risk Exposure Scenarios

Middleton et al. (2022)
Next Steps

- Testing 40+ chemicals using the same approach
- Further iterations to ensure the toolbox is protective and useful
- Identify additional or redundant NAMs
Summary

- The ICCR Principles provide a guide to help apply NAM-based approaches to cosmetics risk assessment, but are also applicable to foods.
- A “Protection not Prediction” approach provides a conservative safety decision, assuming relevant bioactivities are covered.
- The NGRA toolbox needs to be broadly applicable to different chemistries, including food contaminants.
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References

- Reynolds et al., A Bayesian approach for inferring global points of departure from transcriptomics data, Comp Tox. 16:100138 doi:10.1016/j.comtox.2020.100138