Using next generation risk assessment to make safety decisions for consumer products

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Outline

• Why is NGRA important?
• What is it?
• How is it being applied today?
• Where next?
The need for non-animal approaches

Societal Attitudes/Consumer Preference

Human Relevance

Regulatory Change
The Systemic Challenge

Is it safe?

A new non-animal paradigm is needed...

...but replacement of animal test data is not the answer

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**Existing approaches**

Threshold of Toxicological Concern
(Yang et al 2017)
https://doi.org/10.1016/j.fct.2017.08.043

Read across

History of Safe Use
(Neely et al 2011)
https://doi.org/10.4103/0971-6580.85882

→ NGRA
What is NGRA?

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

Dent et al. (2018) Comp Tox 7:20-26
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 transparent 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
https://doi.org/10.1093/toxsci/kfq220
The Margin of Safety Approach

Are in vitro PoDs protective and useful?
Efforts to Reduce Animal Testing at EPA

On September 10, 2015, EPA Administrator Andrew Wheeler signed a directive that prioritizes efforts to reduce animal testing. The memorandum calls for the agency to:
- reduce its requests for, and funding of, animal studies by 30 percent by 2025, and
- eliminate all animal study requests and funding by 2026.

Case Study Approaches... Imagine we have no data for: **Coumarin**

Collection of Existing Data and ADME Parameters

Chemistry determinations:
- Partition coefficient logP
- Peptide binding potential

In vitro determined:
- Kinetic solubility
- Thermodynamic solubility
- Metabolic & chemical stability
- Stability in human plasma
- Plasma protein binding
- Partitioning in blood
- Skin penetration parameters

<table>
<thead>
<tr>
<th>Name</th>
<th>Coumarin</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS</td>
<td>91-64-5</td>
</tr>
<tr>
<td>MW</td>
<td>146.14 g/mol</td>
</tr>
<tr>
<td>Log P</td>
<td>1.39</td>
</tr>
<tr>
<td>Solubility</td>
<td>0.96 mg/mL in phosphate buffer</td>
</tr>
<tr>
<td>ECCS Class</td>
<td>Class 2 (Metabolism)</td>
</tr>
<tr>
<td>Rb2p</td>
<td>0.7</td>
</tr>
<tr>
<td>Fub</td>
<td>0.31</td>
</tr>
<tr>
<td>Clint</td>
<td>929 L/h</td>
</tr>
</tbody>
</table>
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>Parameter</th>
<th>Face cream (applied 2x/day)</th>
<th>Body lotion (applied 2x/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Cmax total (µM)</td>
<td>0.023</td>
<td>0.10</td>
</tr>
<tr>
<td>95th percentile Cmax (µM)</td>
<td>0.032</td>
<td>0.14</td>
</tr>
</tbody>
</table>

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.

Ab Initio NGRA Framework

Exposure Estimation
- Use scenario
- Consumer Habits
- Applied dose
- ADME parameters
- Exposure (PBK)

Collate Existing Information
- Molecular structure
- In silico predictions
- Literature

Local and systemic exposure estimates

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

Risk Assessment Conclusion
- Plasma C_max
  - Sufficient data and high certainty
  - Insufficient data and/or low certainty

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

TIER 0
TIER 1
TIER 2
All binding and enzymatic assay results were negative at 10 µM.

No receptor/target-led pharmacological effect.
**In Vitro Bioactivity: Cell Stress Panel**

Hatherell et al., 2020 Tox Sci 176(1): 11-33 [https://doi.org/10.1093/toxsci/kfaa054](https://doi.org/10.1093/toxsci/kfaa054)

**~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)
- Toglitazone (drug)
- Pioglitazone (drug)
- Rosiglitazone (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).

*now conducted in HepaRG spheroids*
In Vitro Bioactivity: Cell Stress Panel
High-Throughput Transcriptomics Gene Expression Profiling (HTTr)

1. Defining a safe operating exposure for systemic toxicity using a **NOTEL** (No Transcriptional Effect Level)
2. Defining compound similarity grouping (Read Across)

**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

- Coumarin dose range 0.001uM to 100uM
- 24 hour time point
- QC and normalisation in DESeq2
- BMDExpress2 applied to determine NOTEL
  (3 pathway approaches)

<table>
<thead>
<tr>
<th>Cell Model</th>
<th>HepG2</th>
<th>HepaRG</th>
<th>MCF7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pathway Level Tests</strong></td>
<td>(308 pathways)</td>
<td>(0 pathways)</td>
<td>(17 pathways)</td>
</tr>
<tr>
<td>20 pathways with the lowest p-value Reactome</td>
<td>70</td>
<td>NA</td>
<td>58*</td>
</tr>
<tr>
<td>20 pathways with the lowest BMD Reactome</td>
<td>44</td>
<td>NA</td>
<td>58*</td>
</tr>
<tr>
<td>BMD of Reactome pathway with lowest BMD that meets significance threshold criteria</td>
<td>31</td>
<td>NA</td>
<td>38</td>
</tr>
<tr>
<td><strong>Gene Level Tests</strong></td>
<td>(1570 genes)</td>
<td>(47 genes)</td>
<td>(87 genes)</td>
</tr>
<tr>
<td>Mean BMD of 20 genes with largest fold change</td>
<td>6</td>
<td>3</td>
<td>54</td>
</tr>
<tr>
<td>Mean BMD of Genes between 25th and 75th percentile</td>
<td>17</td>
<td>1</td>
<td>59</td>
</tr>
</tbody>
</table>
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 or bioactivity:exposure ratio 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>
</tr>
</thead>
<tbody>
<tr>
<td>Cell stress panel</td>
<td>HepG2 [ATP, 24h]</td>
<td>96738</td>
<td>22048</td>
</tr>
<tr>
<td>Cell stress panel</td>
<td>NHEK [OCR 1h]</td>
<td>1330</td>
<td>295</td>
</tr>
<tr>
<td>HTTr</td>
<td>HepG2 [24h]</td>
<td>7223</td>
<td>1618</td>
</tr>
<tr>
<td>HTTr</td>
<td>HepaRG [24h]</td>
<td>8864</td>
<td>1986</td>
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<tr>
<td>Toxcast</td>
<td>MAO B [rat bain]</td>
<td>3711</td>
<td>831</td>
</tr>
<tr>
<td>PubChem</td>
<td>Carbonic Anhydrase Type I</td>
<td>706</td>
<td>158</td>
</tr>
<tr>
<td>PubChem</td>
<td>Carbonic Anhydrase Type II</td>
<td>2140</td>
<td>479</td>
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<tr>
<td>PubChem</td>
<td>Carbonic Anhydrase Type VI</td>
<td>14652</td>
<td>3282</td>
</tr>
<tr>
<td>Cell stress panel</td>
<td>HepaRG_3D (cell mem perm 168h)</td>
<td>9601</td>
<td>1927</td>
</tr>
<tr>
<td>HTTr</td>
<td>HepaRG_3D_24h</td>
<td>9538</td>
<td>2137</td>
</tr>
</tbody>
</table>
Broader application and acceptance

Case Study on use of an Integrated Approach for Testing and Assessment (IATA) for Systemic Toxicity of Phenoxyethanol when included at 1% in a body lotion

Series on Testing and Assessment, No. 349
Evaluating the level of protection

Chemical exposures scenarios

- ‘Low’ risk (from consumer goods perspective) – e.g. foods, cosmetics
- ‘High’ risk (from consumer goods perspective) – e.g. drugs

Bioactivity:Exposure Ratio (BER)

Define typical use-case scenarios benchmark chemical-exposures

Calculate BER

PBK models of systemic exposure

Calculate the PoDs
Evaluating the level of protection

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- ‘Low’ risk (from consumer goods perspective) – e.g. foods, cosmetics
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PBK models of systemic exposure

Calculate the PoDs

Calculate BER

Bioactivity:Exposure Ratio (BER)
Where next?

• Clarity on the level of protection offered by this approach
  • Bioactivity vs. Adversity
• Adequacy of cell lines, timepoints, study designs – what to do when the ‘protective not predictive’ NGRA fails
• Role of metabolism
• Translating principles to other sectors/chemistries
  • Regulation keeping pace with science
Conclusions

• We are seeing increased pace of development and application of next generation risk assessments in the consumer products industry

• NGRA is exposure-led, hypothesis driven, and requires clear articulation of the risk assessment question

• Progress has been possible with a change in mindset (protection not prediction)

• Once we understand the strengths and limitations why shouldn’t the same approach be useful in different contexts?
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