NAMs for use in NGRA for Systemic Safety: A pragmatic approach to ‘validation’ by establishing protectiveness and utility

Paul Carmichael
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Ensuring Safe Ingredients for Foods, Drinks, Homecare and Cosmetic Products (not drugs)

Risk Based Approach:
Considers both the hazard and the exposure to evaluate the risk.

Can we safely use % of ingredient in product?

For consumers; workers; the environment.
The Need for Implementation of NAM-Based Safety Assessments

COSMETICS
Animal testing bans since 1998

Human relevance

Resource constraints

Societal attitudes/consumer preference

Regulatory change (e.g., EU Cosmetic Regulation)
Non-Animal Protective Frameworks for Safety Decisions

Development of battery of assays aligned to AOPs

AOPs (currently 470 in AOP wiki)

~ Multiple 1000s of assays need to be if multiple AOPs are covered

How to identify the relevant AOP?

Not feasible as a Tier 1 approach

Useful for Tier 2/bespoke safety assessment when differentiation between bioactivity & adversity is needed

Development of high-throughput & broad coverage set of non-animal NAMs

Protection Hypothesis:

If biological activity measured using a broad suite of human-relevant test systems is above the predicted exposure in humans, then systemic adverse effects are highly unlikely
The EPA Blueprint

Tier 1

- Chemical Structure and Properties
  - No Defined Biological Target or Pathway
- Broad Coverage, High Content Assay(s)
  - Defined Biological Target or Pathway
- Multiple cell types +/- metabolic competence

Tier 2

- Select In Vitro Assays
  - Orthogonal confirmation

Tier 3

- Existing AOP
  - In Vitro Assays for other KEs and Systems Modeling
  - Estimate Point-of-Departure Based on Biological Pathway or Cellular Phenotype Perturbation
- No AOP
  - Organotypic Assays and Microphysiological Systems
  - Estimate Point-of-Departure Based on AOP
  - Estimate Point-of-Departure Based on Likely Tissue- or Organ-level Effect without AOP
  - Identify Likely Tissue, Organ, or Organism Effect and Susceptible Populations
Next Generation Risk Assessment (NGRA) – Protection not Prediction

Distributions of Oral Equivalent Values and Predicted Chronic Exposures

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

Safety margin

Actual Exposure (est. max.)

If there is no bioactivity observed at consumer-relevant concentrations, there should be no adverse health effects

Slide from Dr Rusty Thomas, EPA, with thanks


How Protective are those NAMs?  
Example from the Accelerating the Pace of Chemical Risk Assessment (APCRA) initiative – a ‘validation’ of Protection not Prediction?

Of the 448 substances, ~90% had a POD\textsubscript{NAM,95} that was less than the traditional POD (POD\textsubscript{traditional}) value

**Bioactivity:exposure ratios (BERs)**, useful for identification of priority substances, demonstrated that high-throughput exposure predictions were greater than the POD\textsubscript{NAM,95} for 11 substances
EPA Transition from ToxCast to Broad Coverage NAM ‘Product’

High throughput profiling (HTP) assays are proposed as the first tier in a NAMs-based hazard evaluation approach.

HTP Assay Criteria:
1. Yield bioactivity profiles that can be used for potency estimation, mechanistic prediction and evaluation of chemical similarity
2. Compatible with multiple human-derived culture models
3. Concentration-response screening mode
4. Potential to detect specific and non-specific bioactivity

To date, EPA has identified and implemented two HTP assays that meet this criteria:
- **High-Throughput Transcriptomics [HTTr]**
- **High-Throughput Phenotypic Profiling [HTPP]**
**Exposure Assessment** → **Bioactivity-Exposure Characterization** → **Bioactivity Assessment**

- **Expocast (Pop median)** + **httk-pop (Pop distrb’n)** → **Bioactivity-Exposure Ratio**
  - 95th percentile
  - C_{ss}
  - Most sensitive or 5th percentile
  - AC50
  - **HTTr/HTPP**

- **Use-scenario(s)** + **Gastro+ (Pop average)** → **Bioactivity-Exposure Ratio Distribution**
  - Pop average
  - C_{max} distribution
  - Most sensitive platform POD
  - **NGRA panel: CSP + IPP + HTTr**

- **Use-scenario(s)** + **Gastro+ (Pop average)** → **“Sensitive” Individual Bioactivity-Exposure Ratio Distribution**
  - Pop average
  - C_{max} distribution
  - Most sensitive phenotype POD
  - **5 human cell types**
Building NAMs/NGRA Confidence: End-to-End Case Studies

≈40 compounds

448 compounds

46 compounds

30 compounds

>22 compounds

Utility of In Vitro Bioactivity as a Lower Bound Estimate of In Vivo Adverse Effect Levels and in Risk-Based Prioritization

From visions toward best practices: Evaluating in vitro transcriptomic points of departure for application in risk assessment using a uniform workflow
NAMs/NGRA Framework Approach: The overall goal is a human safety risk assessment

TIER 0: Problem Formulation
- Characterise the chemical
- Characterise the consumer exposure scenario
- Collate all available information (literature mining)

Exit if safety decision can be made

Use of predictive tools (i.e. in silico QSAR models)
- Use of exposure-based waiving approaches such as Threshold of Toxicological Concern (TTC)
- Read Across

Exit if safety decision can be made

TIER 1: Data Generation
- Exposure refinement including generation of relevant ADME parameter data for PBK model development
- Bioactivity data generation

Calculate BER

Exit if safety decision can be made

TIER 2: Refine Assessment to Increase Decision Certainty
- Bespoke assays to cover remaining uncertainties identified in Tier 0 or Tier 1
- Further exposure refinement, e.g. consideration of transporters, metabolism, etc...

Exit Safety Decision

TIER 0:
- Problem Formulation

TIER 1:
- Data Generation

TIER 2:
- Refine Assessment to Increase Decision Certainty

Bioactivity data generation
- Concentration [µM]
- Time
- Plasma Cmax
- Lowest platform PoD

Concentration [µM]
- Progress if safety decision can’t be made
- Exit if safety decision can’t be made

Progress if safety decision can’t be made

Exit if safety decision can’t be made
NAMs/NGRA Framework Approach:
The overall goal is a human safety risk assessment

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- Exit Safety Decision

**Early Tier Systemic Toolbox**
- Concentration [µM]
- Control data
- Time
- Plasma Cmax
- Lowest platform PoD
- NAMs/NGRA Framework Approach:
  - The overall goal is a human safety risk assessment
  - Early Tier Systemic Toolbox
  - TIER 0: Problem Formulation
  - TIER 1: Data Generation
  - TIER 2: Refine Assessment to Increase Decision Certainty
A framework for establishing scientific confidence in new approach methodologies

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Evaluation/“Validation” of an Early Tier Toolbox for Systemic Safety

**AIM:** Use NAMs to ensure the protection of consumers: can the approach be used to confidently identify low risk chemical exposure scenarios?

1. **Define the toolbox components** Choose and evaluate a set of NAMs covering exposure modelling and bioactivity investigations

2. **Select test chemicals** Choose as many as practicable to maximise coverage of different chemistries and biological effects/toxicity

3. **Set performance criteria** Define the ‘truth’ that the performance of the toolbox will be compared to
Our Key NAMs

PBK Modelling

In vitro pharmacological profiling

Transcriptomics

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

Cellular Stress Pathways

13 chemicals, 36 Biomarkers; 2 Timepoints; 8 Concentrations; ~ 10 Stress Pathways

Toxicol Sci (2020), 176, 11-33

Toxicology in Vitro (2020), 63, 104746
1. Defining the Toolbox Components

Point of Departure Determination

**Nonselective Effects**
- Transcriptomics
  - Use of full human gene panel
  - 21k
  - 24 hrs exposure
  - 7 concentrations
  - 3 cell lines: HepG2/HepaRG/MC3T
  - 3D HepaRG spheroids

- BMDerived 2

**Cellular Stress Pathways**
- 13 chemicals: 36 Biomarkers; 3 Timepoints; 8 Concentrations; 16 Stress Pathways

**Selective Effects**
- In vitro pharmacological profiling

**Bioactivity Exposure Ratio Distribution**

**PBK Modelling**
- Plasma \(C_{\text{max}}\) estimate
- \(C_{\text{max}}\)- Error Distribution model (CMED) (Bayesian model)
2. Select Test Chemicals

Collate possible chemicals from databases, large-scale projects, expert opinion

Filter out chemicals that would be impractical to test

38 Test Chemicals

- 9 cosmetic ingredients, 21 drugs, 3 food additives, 5 agricultural chemicals, 1 industrial chemical

- Oral, dermal, IV and inhalation exposure scenarios

- Organ toxicities, CNS disruptions, immune system dysregulation, non-specific effects, blood-based disorders etc...
3. Set Performance Criteria

Benchmarking using chemical-exposure scenarios

- Chemicals with well-defined human exposures
- Traditional safety assessment available
- High certainty in the risk classification for each chemical-exposure scenario from a consumer goods perspective
- Risk class is relative to consumer health (N.B. drugs = high-risk)

‘Low’ risk for consumers from systemic perspective

‘High’ risk for consumers from systemic perspective

How many of the high-risk exposure scenarios are identified as uncertain/high risk? (i.e. BER < threshold)

How many of the low-risk scenarios are identified as low-risk at this early tier stage in a risk assessment framework? (i.e. BER > threshold)
3. Set Performance Criteria

Benchmarking using chemical-exposure scenarios

- Chemicals with well-defined human exposures
- Traditional safety assessment available
- High certainty in the risk classification for each chemical-exposure scenario from a consumer goods perspective
- Risk class is relative to consumer health (N.B. drugs = high-risk)

'Low' risk for consumers from systemic perspective

'High' risk for consumers from systemic perspective

Threshold values of the BER point estimates for determining whether an exposure is low risk

<table>
<thead>
<tr>
<th>PBK Level</th>
<th>Threshold BER Required for Exposure to Be Identified as Low Risk</th>
<th>Confidence Threshold (pthreshold) Required for Exposure Scenario to Be Identified as Low Risk</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>110</td>
<td>.98</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>.97</td>
</tr>
<tr>
<td>3</td>
<td>2.5</td>
<td>.95</td>
</tr>
</tbody>
</table>
Results for 38 Test Chemicals and 70 Exposure Scenarios

Protectiveness 98% (45 out of 46)

High-risk exposure scenarios are identified as uncertain/high risk (i.e. BER < threshold)

Utility 33% (8 out of 24)

Low-risk scenarios are identified as low risk at this early tier stage in a risk assessment framework (i.e. BER > threshold)
Comparison of NAM-based Early Tier Toolbox with Decisions Made Using \textit{in vivo} Data – Protective not Predictive

What if we took the same approach with \textit{in vivo} data?

- Repeat dose \textit{in vivo} data identified for 27 chemicals of the 38 tested.
- In most cases NAM PoDs are more conservative than traditional PoDs

\textit{In agreement with Paul-Friedman et al (2020)}
Comparison of NAM-based Early Tier Toolbox with Decisions Made Using \textit{in vivo} Data

Using the minimum of NOAELs/LOAELs identified, margins of safety plotted and threshold at MoS = 100
Adverse effects in traditional and alternative toxicity tests

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Current Toxicity Testing Paradigm

- Systemic In Vivo Toxicity Tests
- Mechanism/Specific Endpoint Tests

Integrated Combination of In Vivo Tests

- Non-Specific
- Specific

- Systemic Endpoint-Based Assessment
- MOA/Specific Hazard-Based Assessment

Protective
Predictive

NAM-Based Toxicity Testing Paradigm

- Broad Coverage Technologies/Models
- Target-Specific Technologies/Models

Integrated Combination of Technologies and Models (i.e., iATA)

- Non-Specific
- Specific

- Bioactivity-Based Assessment
- AOP/MOA-Based Assessment

Protective
Predictive
Reproducibility of HepG2 BIFROST global PoD from HTTr

Subset of toolbox evaluation chemicals tested in HepG2 cell line at Unilever + US-EPA

Can compare global PoDs estimated from SEAC and EPA datasets

Moderate correlation (0.82), hampered by noticeable outliers:

**Aspartame** – Has a retinoic acid like signal, suspected contamination in EPA data due to proximity on dosing plate

**Ketoconazole** – Difference attributable to BIFROST modelling choices
Conclusions and Next Steps

• For the test chemicals in this evaluation, an early tier systemic toolbox is **98% protective**

• Fair to say ‘overly-conservative’?
  
  Low utility requires higher-tier tools for bioactivity distinguishing adversity from adaption (AOP and prediction-lead e.g. from ONTOX/RiskHunt3R)

• A NAM-based toolbox for systemic toxicity has comparable performance to safety decision making using traditional *in vivo* data.

• What is the applicability domain of this toolbox?
  
  How would the toolbox perform with a wider set of chemicals?

• What would the performance be like with a different set of assays/cells?
  
  Is there an optimum combination of NAMs to maximise both protectiveness and utility?

• **Assuring human safety is the most important thing**
EPA and Unilever Announce Major Research Collaboration to Advance Non-animal Approaches for Chemical Risk Assessment

August 23, 2021

Contact Information:
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WASHINGTON - Today, the U.S. Environmental Protection Agency (EPA) and Unilever announced a collaborative agreement to explore better ways to assess chemical risks associated with consumer products. This agreement builds upon prior cooperation between EPA and Unilever regarding New Approach Methods (NAMs), which are promising alternatives to conventional toxicity testing that are intended to reduce reliance on the use of animals.

EPA and Unilever have been jointly evaluating and using NAMs since 2016. This collaboration is helping EPA implement its New Approach Methods Risk Plan and is the foundation for new efforts to demonstrate that these novel approaches can help decision makers better protect consumers, workers and the environment.

"EPA is a pioneer in developing and applying NAMs to identify and quantify risks to human health, while reducing the use of animals in preclinical toxicity testing," said M. Christopher Przybylo, Deputy Assistant Administrator for Science Policy in EPA's Office of Research and Development. "We are excited to continue the collaboration with Unilever, which enhances the robustness of our mutual research to demonstrate the use of NAMs."
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