SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety

Alternative Toxicological Approaches for Process-Formed Constituents in Food

January 17, 18, or 19, 2023

Next Generation Systemic Toolbox

Matt Dent, PhD
Unilever Safety and Environmental Assurance Centre
Matthew.dent@unilever.com

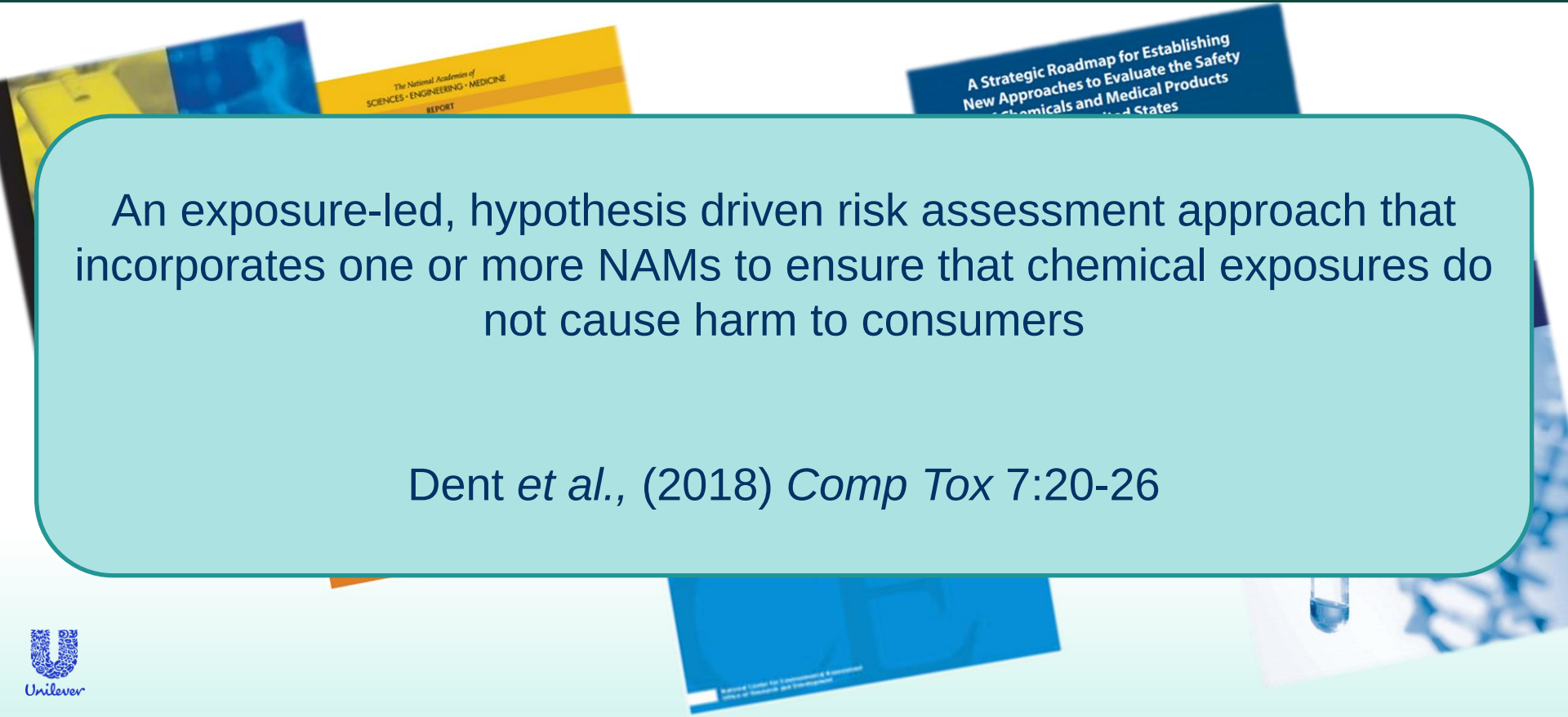
Conflict of Interest Statement

- 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

What is Next Generation Risk Assessment?



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

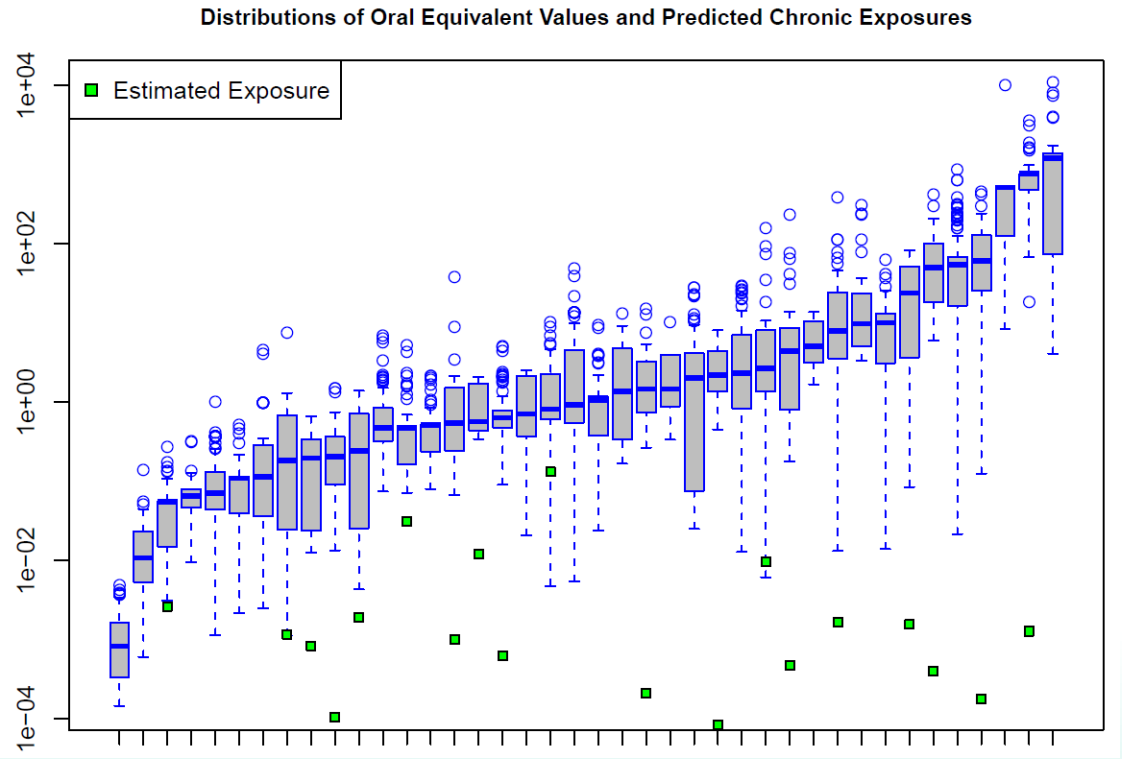
Dent *et al.*, (2018) *Comp Tox* 7:20-26

Unilever

Principles of NGRA

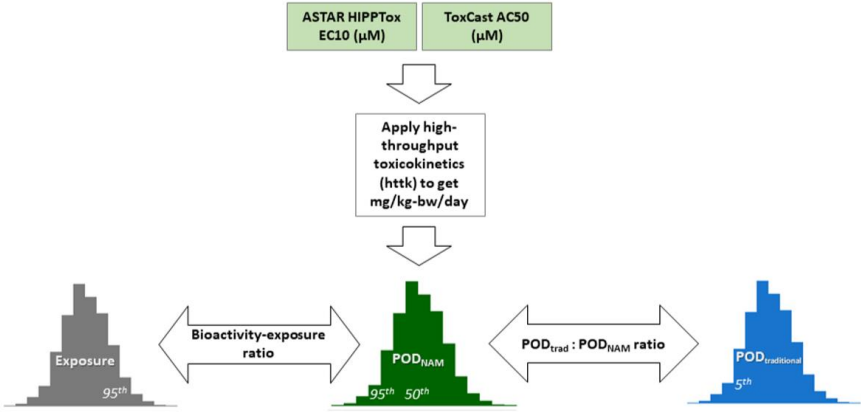
- **Main overriding principles**:
  - The overall goal is a human safety risk assessment
  - The assessment is exposure led
  - The assessment is hypothesis driven
  - The assessment is designed to prevent harm
- **Principles describe how a NGRA should be conducted**:
  - Following an appropriate appraisal 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

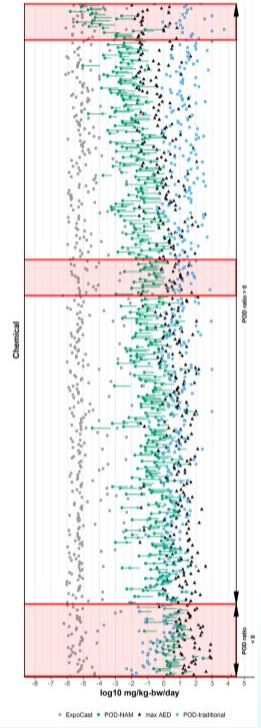
“Protection not Prediction”



Rotroff et al. 2010

EPA, NTP, HC, A*STAR, ECHA, EFSA, JRC, RVIM…





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

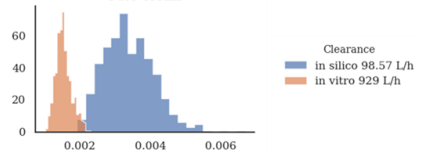
Katie Paul-Friedman *et al.* (2019)

The core NAMs in our systemic NGRA toolbox

Genetic Tox

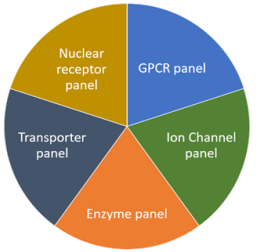
ToxTracker/
Ames/In vitro
Micronucleus

PBK Modelling



Moxon et al., 2020

In vitro pharmacological profiling

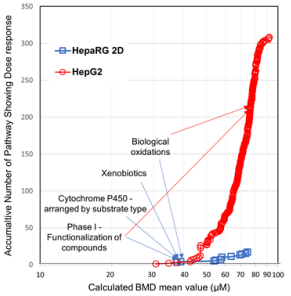


Bowes et al., 2012

Transcriptomics

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

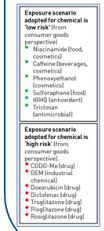
BMDExpress 2

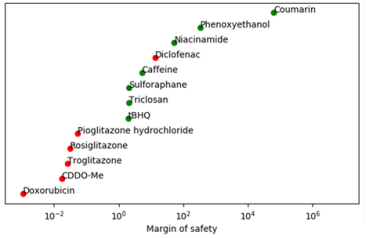


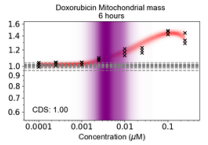
Reynolds et al., 2020

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?

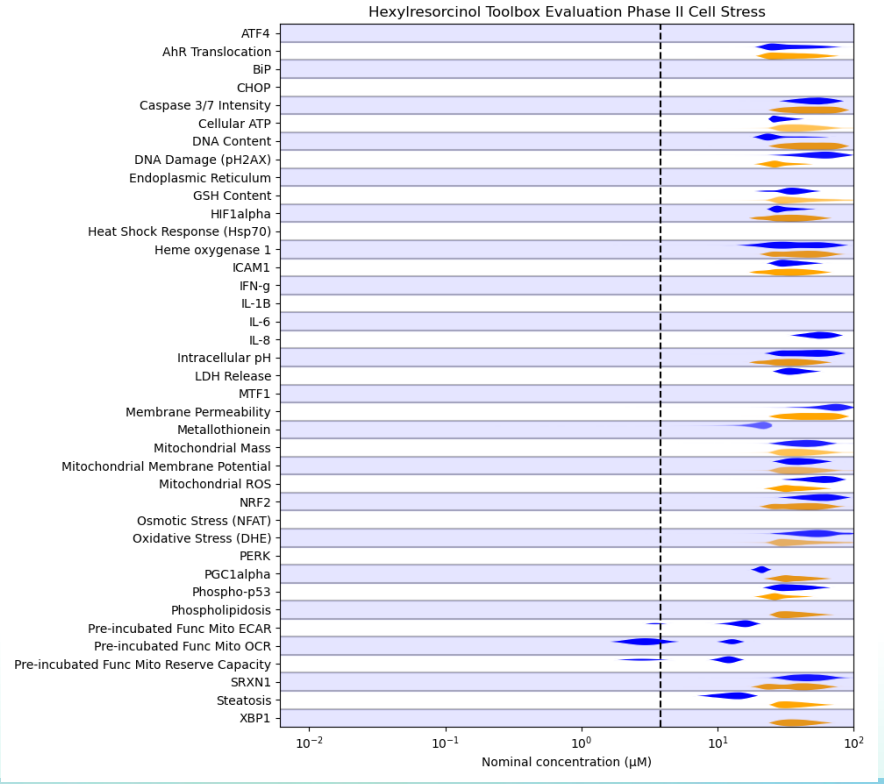
Tiered Approach to Exposure Estimation

- 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_{max} = 0.007 µM
- Level 2: PBK model built with *in vitro* parameters
  - Predicted plasma C_{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)

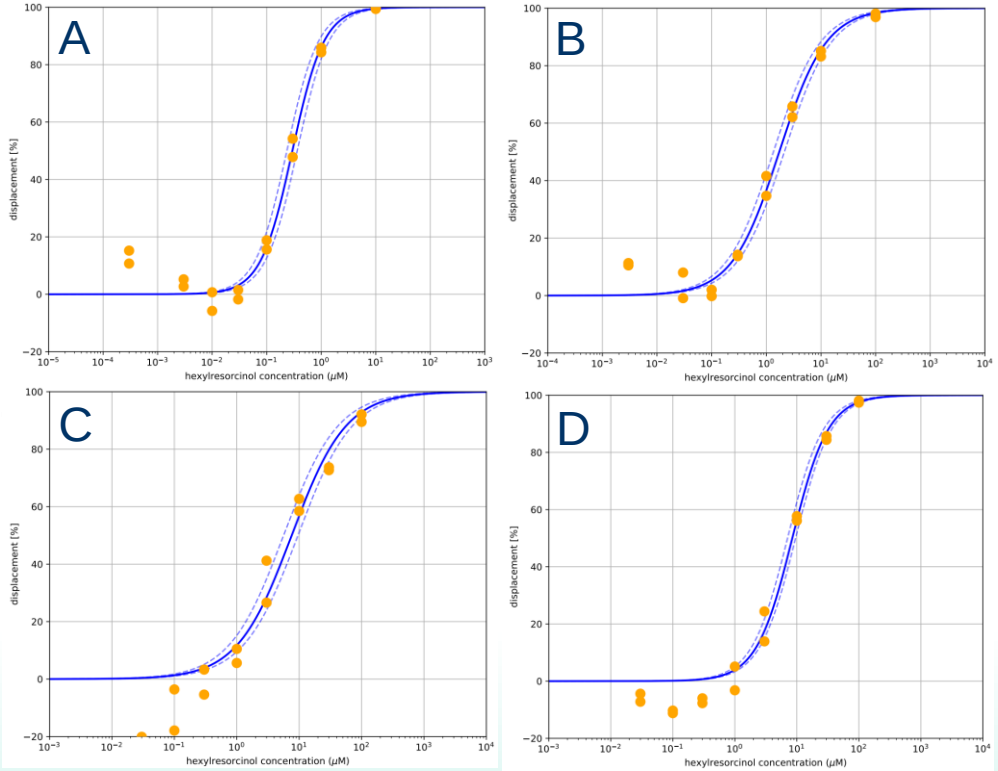
Cell Stress Global Point of Departure = 3.8 µM



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

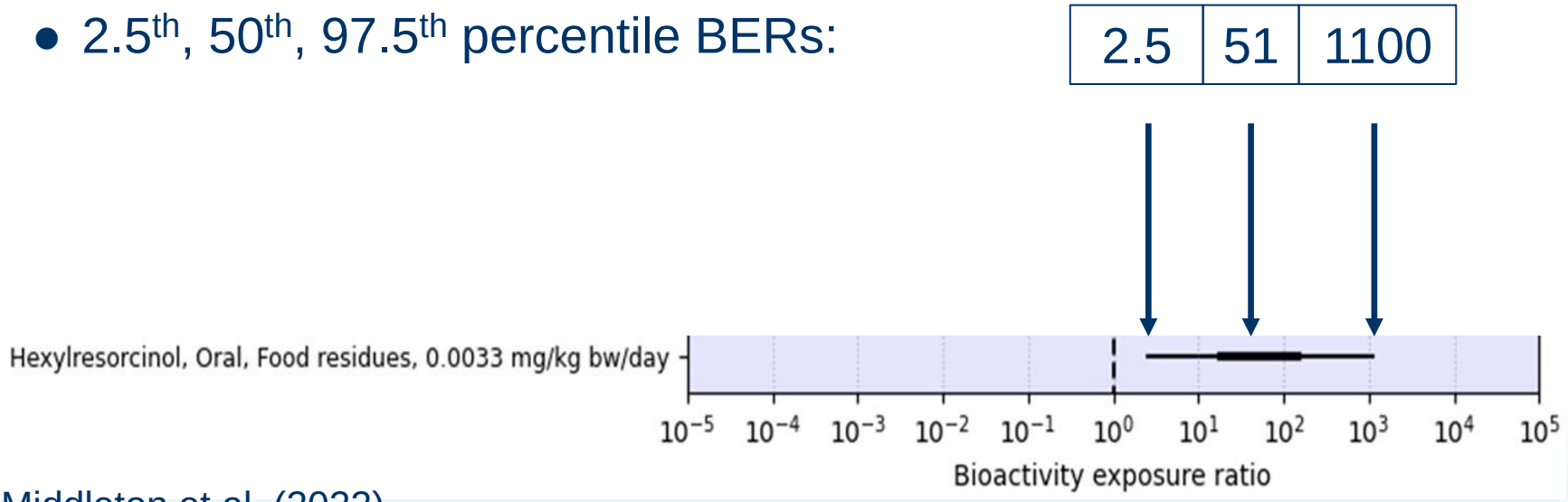
- High throughput transcriptomics data analysed using 2 methods:
  - BIFROST (Bayesian inference for region of signal threshold): Minimum effect concentration across all genes.
  - Benchmark Dose Lower Confidence Interval ($BMDL_{10}$)

Cell Line	Global PoD (µM)	Minimum Pathway BMDL (µM)
HepaRG	8.1	53
HepG2	7.3	27
MCF7	0.8	15

Middleton et al. (2022)

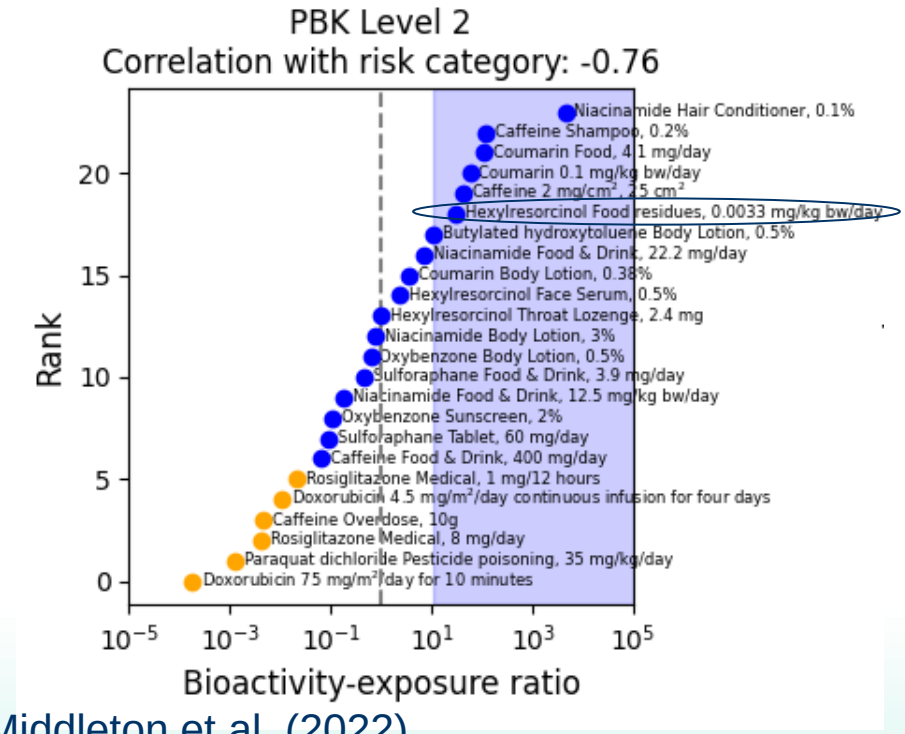
Bioactivity:Exposure Ratio

- Ratio of lowest PoD and Exposure
- 2.5th, 50th, 97.5th percentile BERs:



Middleton et al. (2022)

Toolbox evaluation (pilot phase)

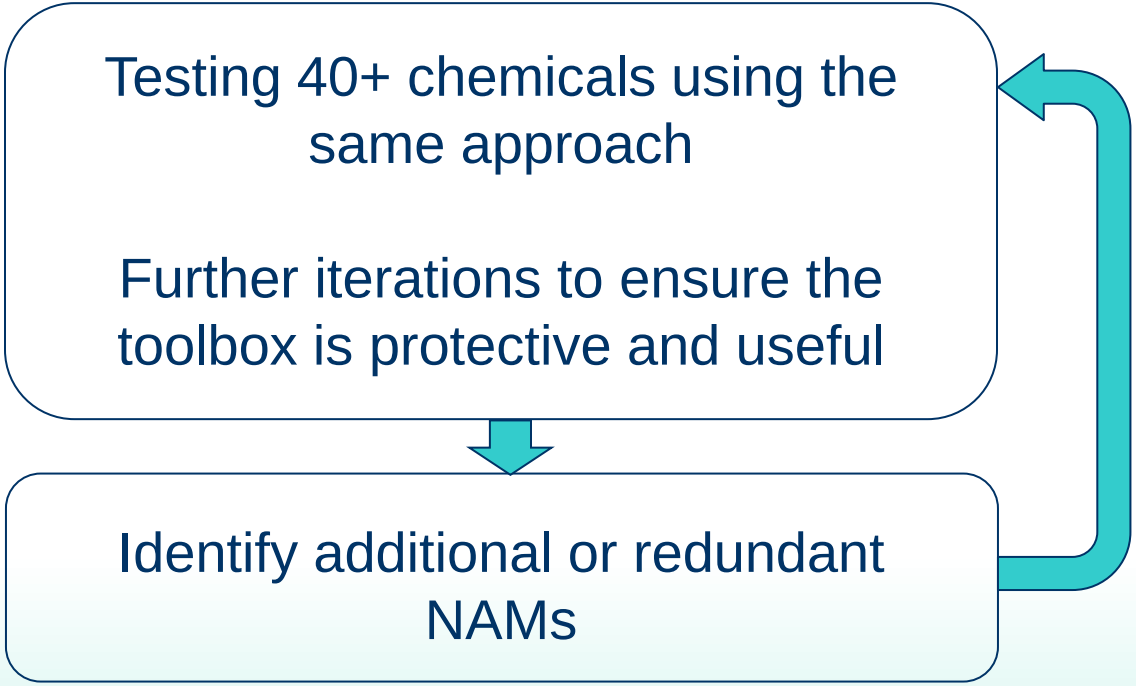


Middleton et al. (2022)

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

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

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

Acknowledgements

- Alistair Middleton, Maria Baltazar, Sophie Cable, Joe Reynolds, Hequn Li , Predrag Kukic, Paul Carmichael, Beate Nicol, Sharon Scott, Sophie Malcomber, Annabel Rigarlsford, Chris Sparham, Trina Barritt, Katarzyna Przybylak, Georgia Reynolds, Andrew White, Sarah Hatherell, Richard Cubberley, Carl Westmoreland, Eurofins, Cyprotex, Bioclavis

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