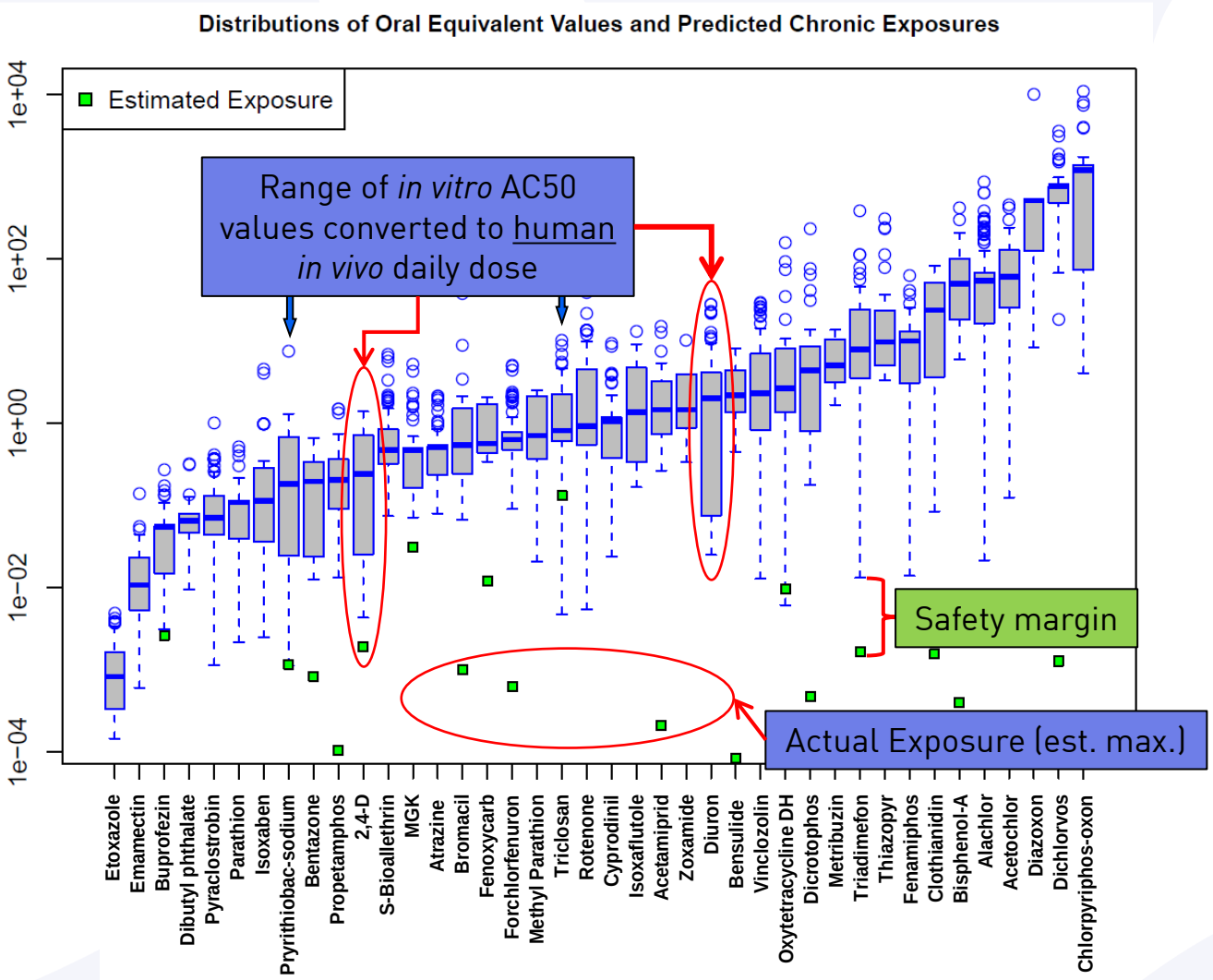
Evaluating New Approach Methodologies for use in Next Generation Risk Assessment

Dr Alistair Middleton
Science leader in Computational Toxicology
Unilever Safety & Environmental Assurance Centre (SEAC)

Middleton et al (2022), *Tox Sci,* Volume 189, Issue 1, Pages 124-147

Paradigm shift for systemic safety - Protection not Prediction



Slide from Dr Rusty Thomas, EPA, with thanks

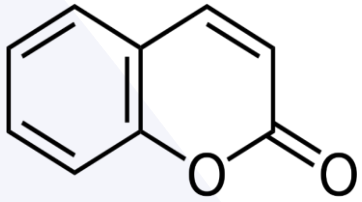
Rotroff, *et al.* Tox.Sci 2010

The hypothesis underpinning this type of NGRA is that **if there is no bioactivity observed at consumer-relevant concentrations, there can be no adverse health effects.**

Thomas RS et al., 2019. Tox Sci. 1;169(2):317-332.

Example how to integrate NAMs for a NGRA: coumarin case study

0.1% COUMARIN IN FACE CREAM AND BODY LOTION (NEW FRAGRANCE)



Plasma C_{max}

Exposure Estimation

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

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
- HTTr – TempO-Seq

Insufficient data and high uncertainty

Metabolism refinement

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

$PoD_{in\ vitro}$

*** Determine Margin of Exposure**

*or Bioactivity exposure ratio (BER)

Sufficient data and high certainty

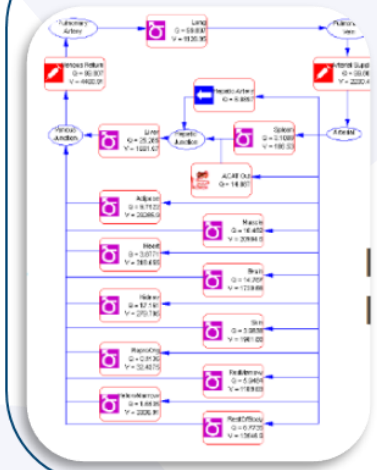
Risk Assessment Conclusion

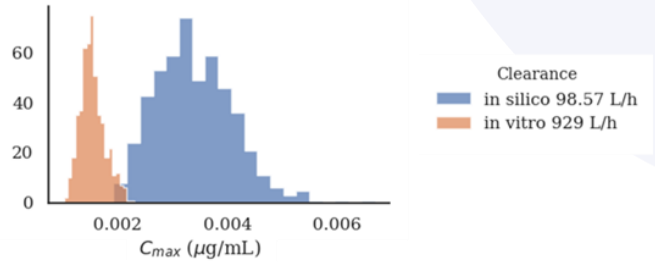
Low risk conclusion based on the margin of safety calculations.

Baltazar *et al.*, (2020) *Tox Sci* Volume 176, Issue 1, 236–252

The key NAMs in our NGRA approach

PBK Modelling





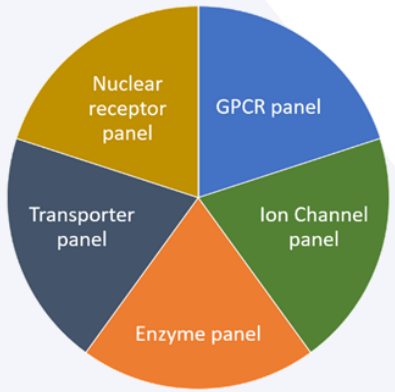
Toxicology in Vitro (2020), **63**, 104746

In vitro pharmacological profiling


PERSPECTIVES

A GUIDE TO DRUG DISCOVERY — OPINION

Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

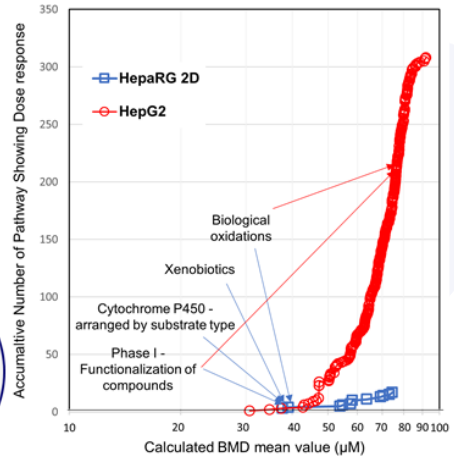


eurofins | Cerep

Transcriptomics

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

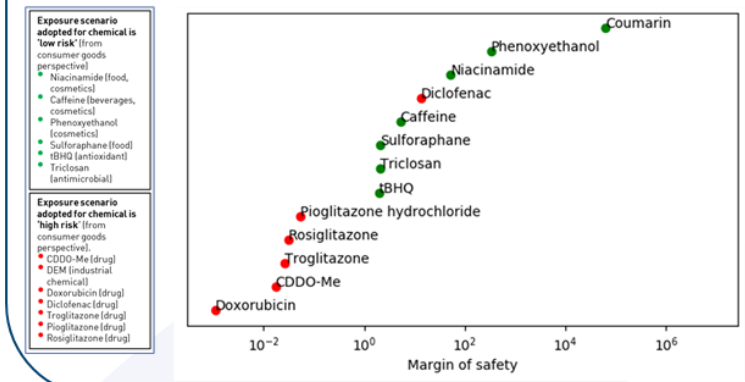
BMDexpress 2

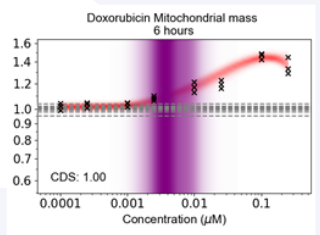


Cellular Stress Pathways

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

cyprotex AN EVOTEC COMPANY





Toxicol Sci (2020), **176**, 11-33

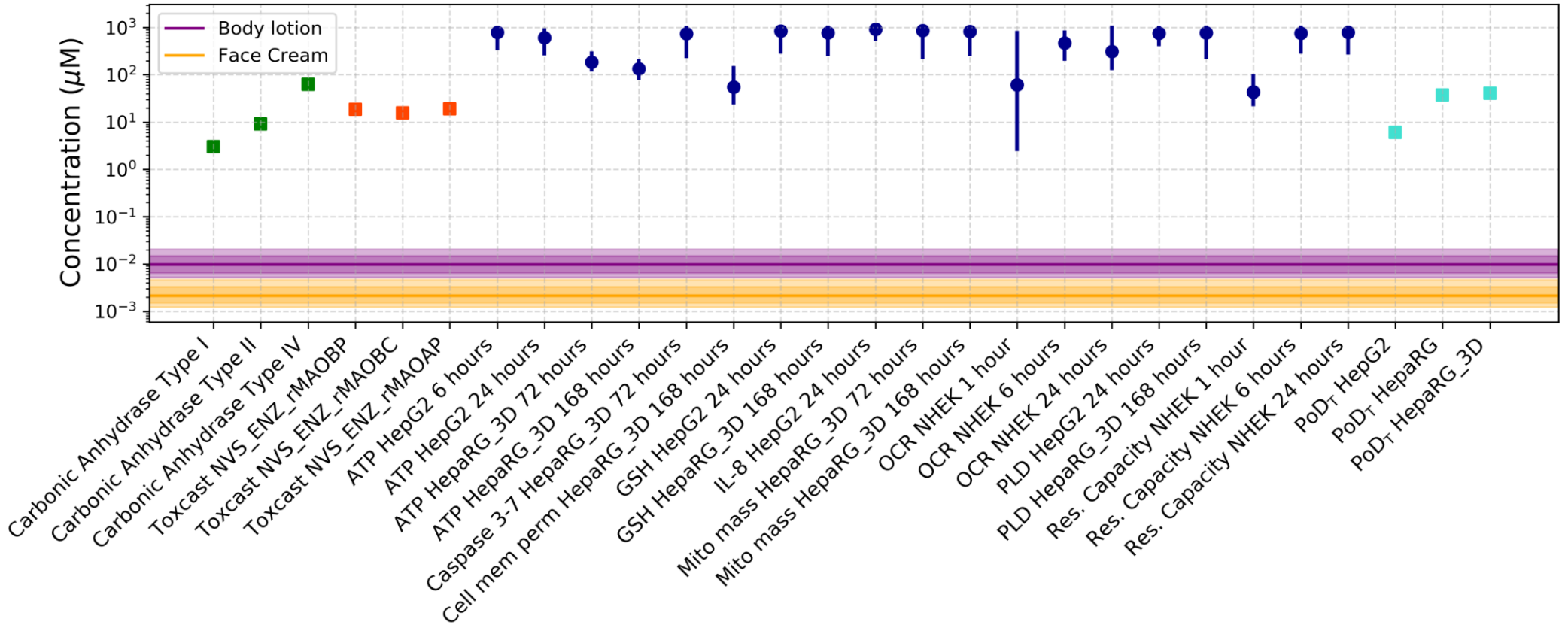
Unilever

Exposure and PoD are plotted and used to derive a Bioactivity-Exposure Ratio (BER)

In-vitro pharmacological profiling (IPP)

Cell Stress Panel

High throughput transcriptomics (HTTR)



The 5th percentile of the BER distribution ranged between 158 and 96738

In this case study: Weight of evidence suggested that the inclusion of 0.1% coumarin in face cream or a body lotion is safe for the consumer

Unilever

How do we build scientific confidence in a systemic safety toolbox?

1. Determine whether the toolbox is fit for purpose (leads to safety decisions that are protective of human health).
2. Take into account human safety in assessing the approach (where possible)
3. Identify what an appropriate safety decision might be (e.g., BER threshold).

Accelerating the Pace of Chemical Risk Assessment (APCRA)

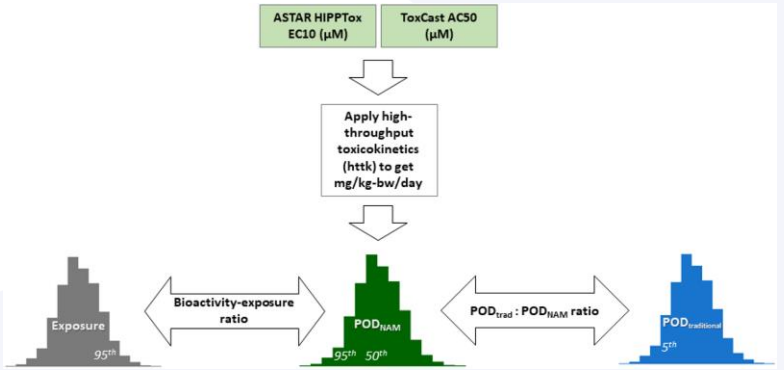
OXFORD SOT Society of Toxicology academic.oup.com/toxsci Tox Spotlight article

TOXICOLOGICAL SCIENCES, 173(1), 2020, 202–225

doi: 10.1093/toxsci/kfz201
Advance Access Publication Date: September 18, 2019
Research Article

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

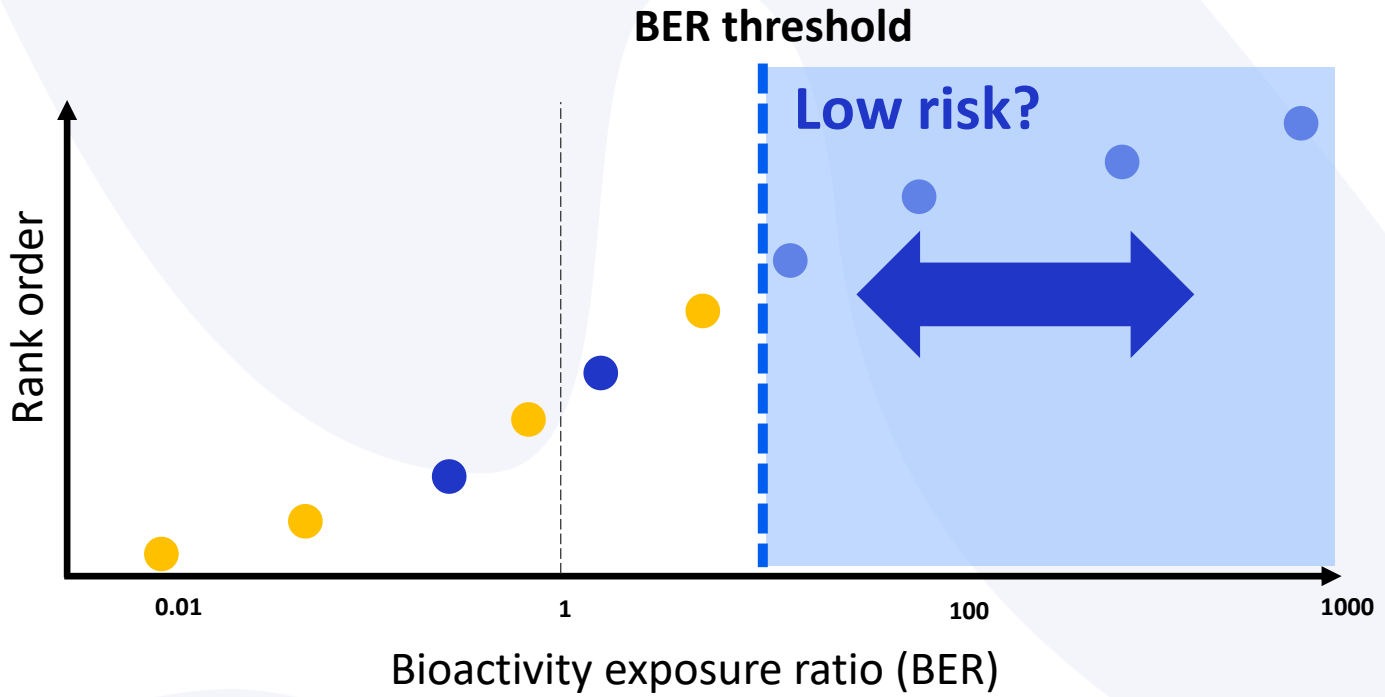
Katie Paul Friedman ,*,[1] Matthew Gagne,[†] Lit-Hsin Loo,[‡] Panagiotis Karamertzanis,[§] Tatiana Netzeva,[§] Tomasz Sobanski,[§] Jill A. Franzosa,[¶] Ann M. Richard,[*] Ryan R. Lougee,[*,||] Andrea Gissi,[§] Jia-Ying Joey Lee,[‡] Michelle Angrish,[|||] Jean Lou Dorne,[||||] Stiven Foster,[#] Kathleen Raffaele,[#] Tina Bahadori,[‖] Maureen R. Gwinn,[*] Jason Lambert,[*] Maurice Whelan,[**] Mike Rasenberg,[§] Tara Barton-Maclaren,[†] and Russell S. Thomas *



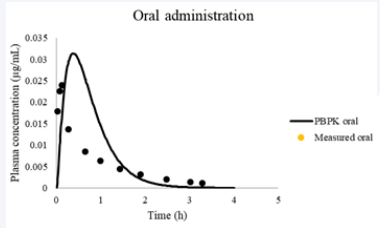
How do we build scientific confidence in a systemic safety toolbox?

Chemical exposures scenarios

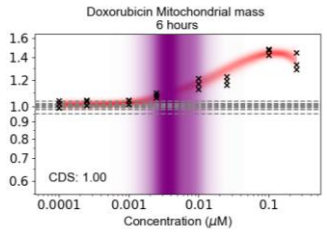
- ‘Low’ risk (from consumer goods perspective) – e.g. foods, cosmetics
- ‘High’ risk (from consumer goods perspective) – e.g. drugs



Define typical use-case scenarios benchmark chemical-exposures; Mixture of High and low risk



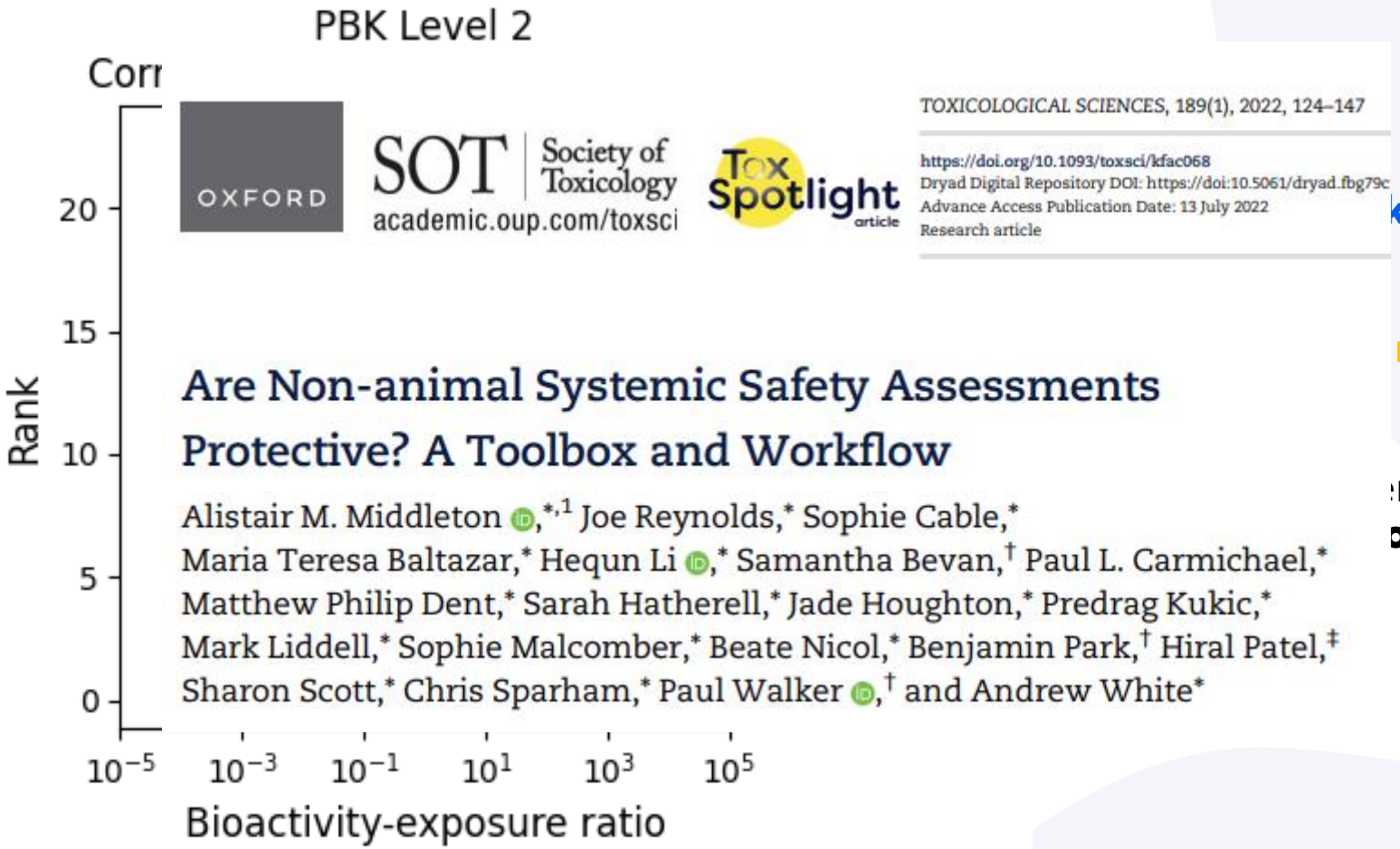
PBK models of systemic exposure



In-vitro cell assays, estimate PoDs

Calculate the bioactivity exposure ratio

Visualising how the toolbox performs against the pilot study data



OXFORD
SOT | Society of Toxicology
academic.oup.com/toxsci
Tox Spotlight article
TOXICOLOGICAL SCIENCES, 189(1), 2022, 124–147
https://doi.org/10.1093/toxsci/kfac068
Dryad Digital Repository DOI: https://doi:10.5061/dryad.fbg79c
Advance Access Publication Date: 13 July 2022
Research article

Are Non-animal Systemic Safety Assessments Protective? A Toolbox and Workflow

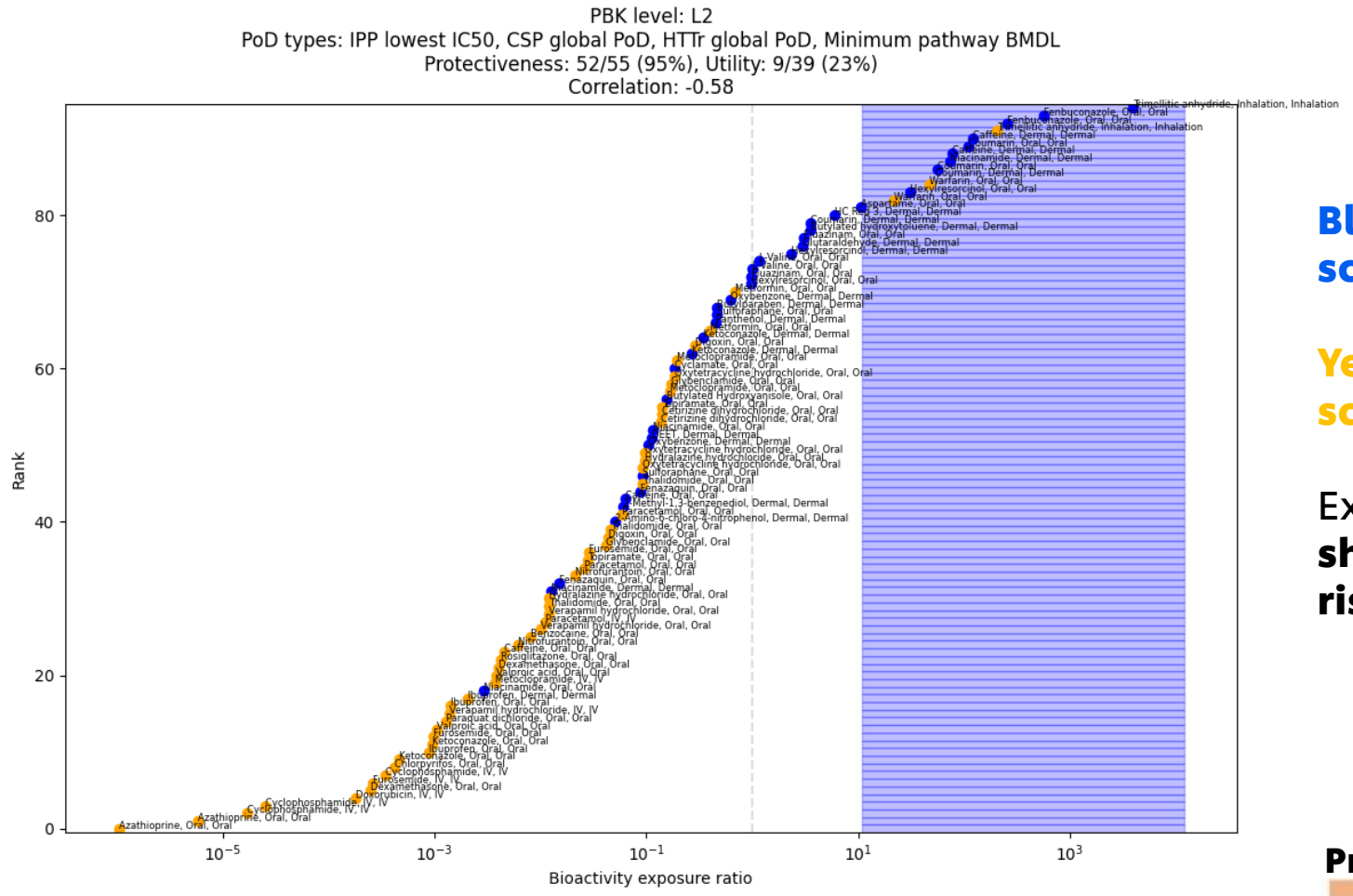
Alistair M. Middleton,*,1 Joe Reynolds,* Sophie Cable,* Maria Teresa Baltazar,* Hequn Li,* Samantha Bevan,† Paul L. Carmichael,* Matthew Philip Dent,* Sarah Hatherell,* Jade Houghton,* Predrag Kukic,* Mark Liddell,* Sophie Malcomber,* Beate Nicol,* Benjamin Park,† Hiral Patel,‡ Sharon Scott,* Chris Sparham,* Paul Walker,† and Andrew White*

k chemical-exposure

risk chemical-exposure

narios within the **blue**
on are identified as **low**

Extending the evaluation to 38 chemicals and 70 exposure scenarios



Blue: low risk chemical-exposure scenario

Yellow: high risk chemical-exposure scenario

Exposure scenarios within the **blue shaded region** are identified as **low risk.**

Protectiveness and utility metrics

$$\text{Protectiveness} = \frac{H_U}{H_U + H_L}$$

$$\text{Utility} = \frac{L_L}{L_L + L_U}$$

H_U - # of high risk exposures identified as uncertain risk
H_L - # of high risk exposures identified as low risk
L_U - # of low risk exposures identified as uncertain risk
L_L - # of low risk exposures identified as low risk

Unilever

Discussion

- Have now extended the evaluation to 38 chemicals with 70 associated **high risk** and **low risk** exposure scenarios.
- Adopt **iterative approach** to evaluating and then identifying potential improvements to the toolbox.
- **Unilever-EPA CRADA**: Generating data for 10 cell lines, using high-throughput transcriptomics and phenotypic profiling.
- The overall objective is to establish the **scientific confidence** that the toolbox is fit for purpose.
- In the process of mapping activities against existing NAM validation criteria (inc van der Zalm (2022) and OECD TG34)

Archives of Toxicology (2022) 96:2865–2879
https://doi.org/10.1007/s00204-022-03365-4

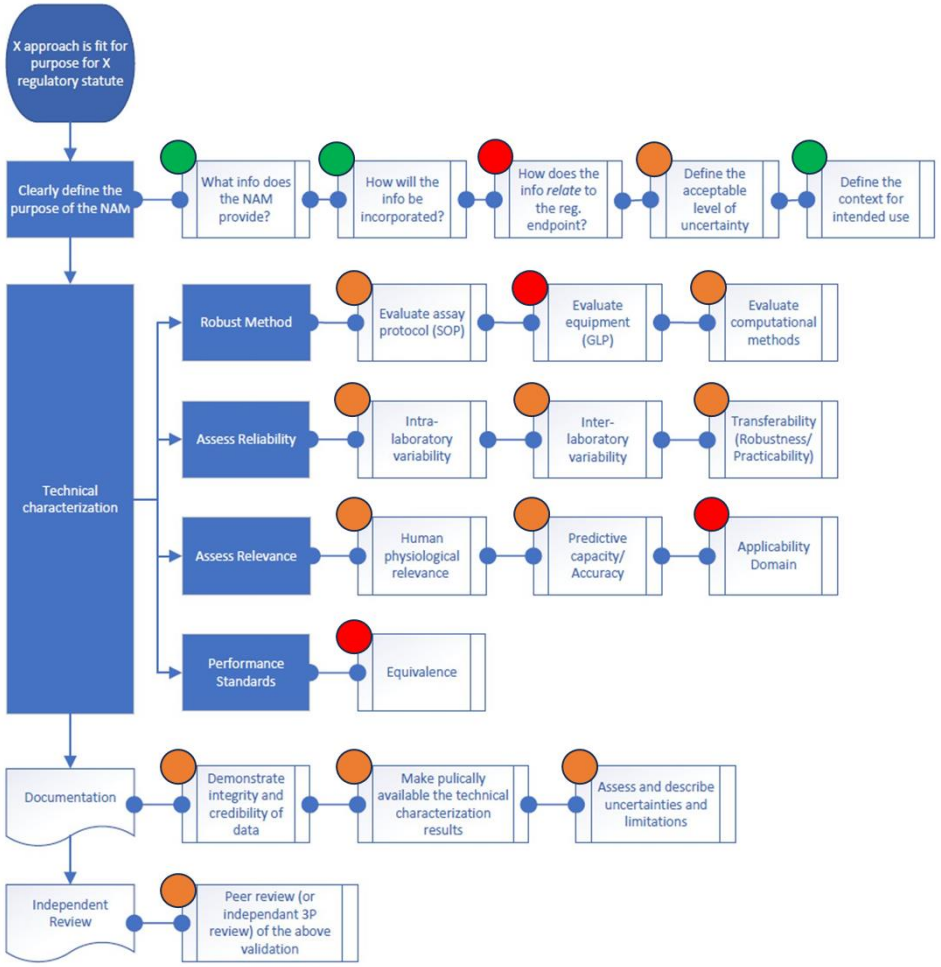
REVIEW ARTICLE

Check for updates

A framework for establishing scientific confidence in new approach methodologies

Anna J. van der Zalm[1] · João Barroso[2] · Patience Browne[3] · Warren Casey[4] · John Gordon[5] · Tala R. Henry[6] · Nicole C. Kleinstreuer[7] · Anna B. Lowit[6] · Monique Perron[8] · Amy J. Clippinger[1]

Received: 17 May 2022 / Accepted: 11 August 2022 / Published online: 20 August 2022



Acknowledgements

Unilever: Maria Baltazar, Sophie Cable , Joe Reynolds, Georgia Reynolds, Beate Nicol, Sharon Scott, Sophie Malcomber, Annabel Rigarlsford, Chris Sparham, Katarzyna Przybylak, Predrag Kukic, Georgia Reynolds, Tom Moxon, Hequn Li, Dawei Tang, Jayasujatha Vethamanickam, Matthew Dent, Andrew White, Paul Carmichael, Sarah Hatherell, Richard Cubberley, Carl Westmoreland

US-EPA: Richard Judson, Josh Harrill, Logan Everett, Imran Shah

Thank You

seac.unilever.com

Unilever

Overall evaluation strategy

Step 1 (pilot study)*

- Define what the toolbox contains (which NAMs) and the workflow through which they should be used.
- Define process of how the toolbox will be evaluated, and the metrics that will be used to determine it's 'performance'
- Explore using a small set of chemicals and exposure scenarios (11 chemicals, 25 exposure scenarios)
- Define **prototype decision model** for determining the BER threshold.

Step 2 (full evaluation)

- Evaluate the toolbox using ~40 chemicals with ~100 exposure scenarios based on the toolbox established in the pilot study.
- Use learnings from the toolbox evaluation to refine the toolbox in terms of **NAM composition** and the **decision model**.

*Middleton et al (2022), *Tox Sci,* Volume 189, Issue 1, Pages 124-147

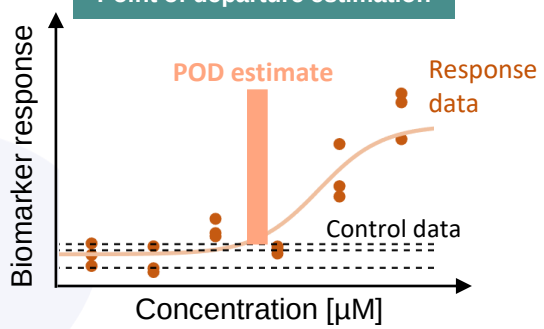
Stage 3: Estimating the BER from the toolbox

POD estimation

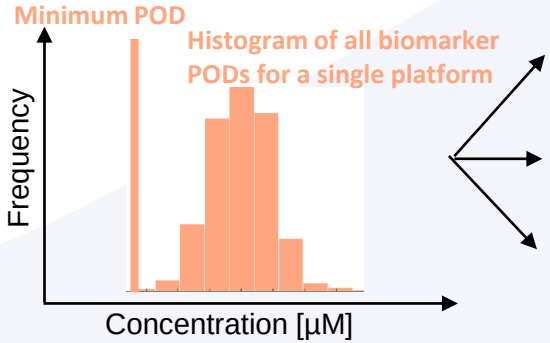
Concentration-response assays

- HTTr (MCF-7, HepG2, HepaRG)
- CSP (HepG2)
- IPP

Point of departure estimation



Summarise biomarker points of departure



- HTTr platform POD (Global POD method or lowest pathway mean BMCL)
- Cell stress platform POD (HepG2)
- IPP platform POD

Minimum platform POD

Exposure estimation

Use-scenario

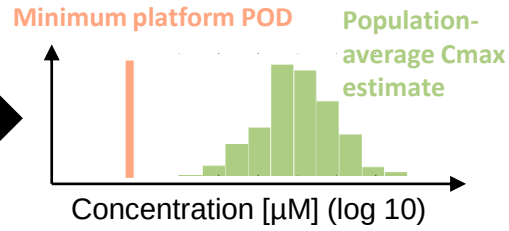
PBK model (Gastroplus)

- *In silico* parameter estimates (L1)
- *In vitro* parameter estimates (L2)
- *Human in vivo PK data* (L3)

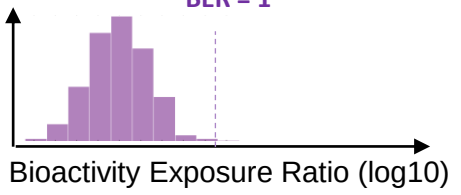
Plasma C_{max} estimate

C_{max} Error Distribution model (CMED) (Bayesian model)

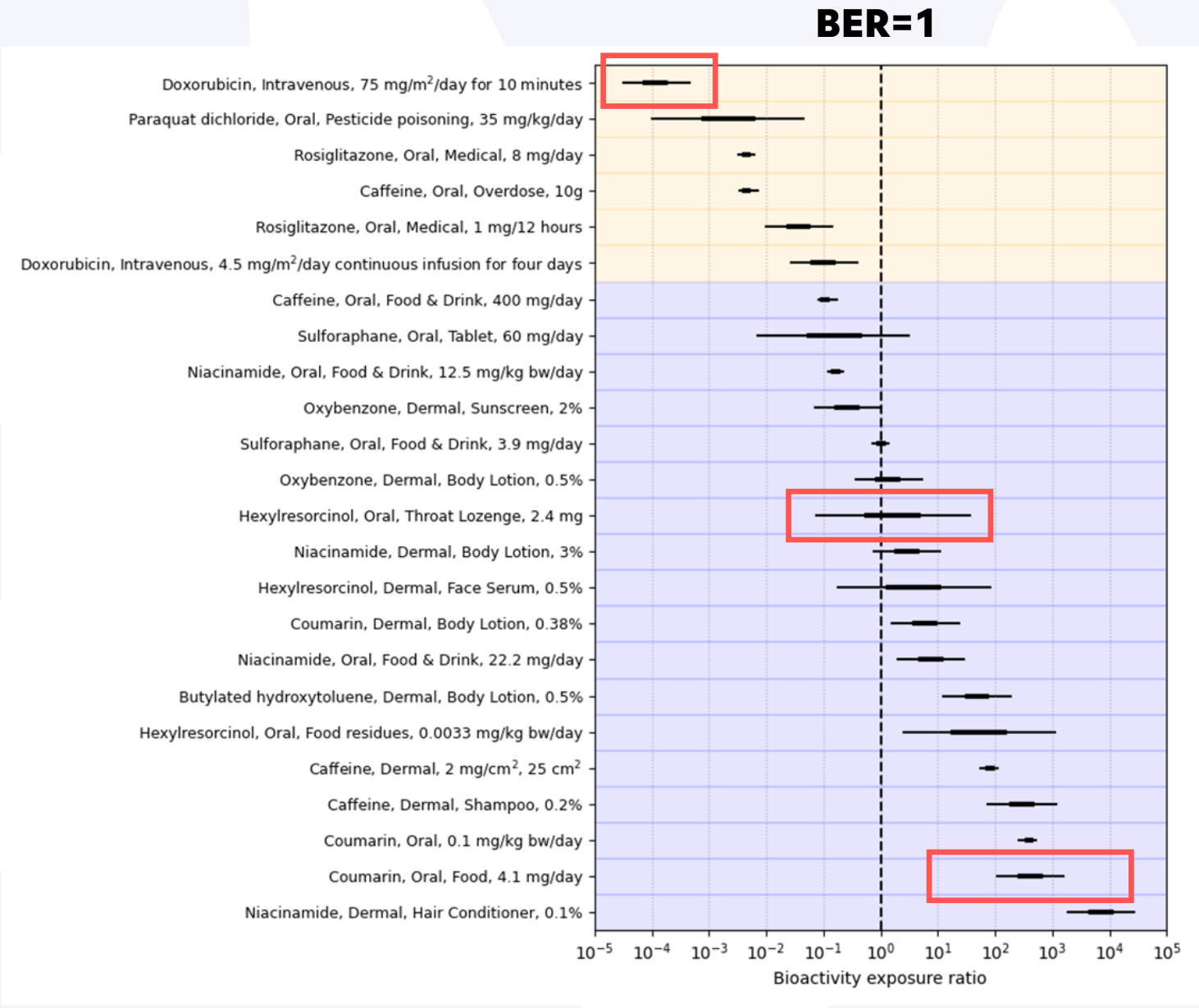
Estimate Bioactivity Exposure Ratio and Decision model



Bioactivity Exposure Ratio Distribution



Stage 3: Estimating the BER from the toolbox



Blue: low risk chemical-exposure scenario

Yellow: high risk chemical-exposure scenario

BER=1:
Cmax estimates coincide with the minimum POD

Unilever

Progress in the application of NAMs in NGRA for systemic safety

NAMs applied in an *ab initio* hypothetical NGRA case study (e.g. coumarin and phenoxyethanol)

OXFORD SOT Society of Toxicology
academic.oup.com/toxsci

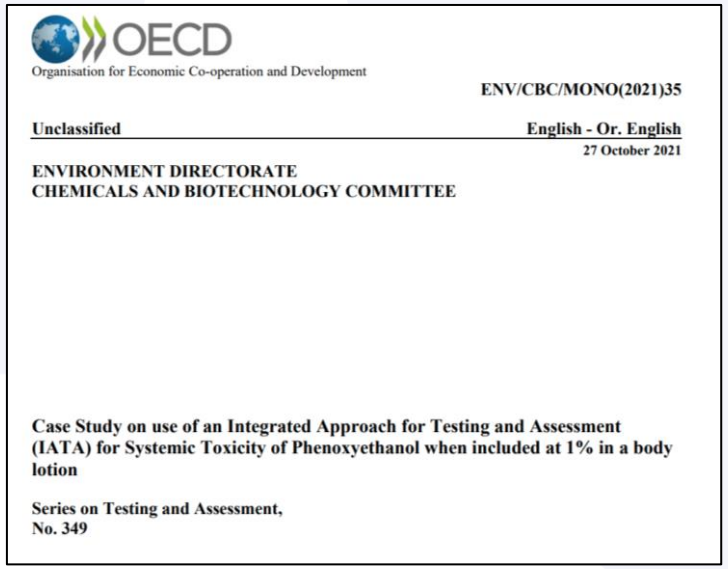
TOXICOLOGICAL SCIENCES, 176(1), 2020, 236–252
doi: 10.1093/toxsci/kfaa048
Advance Access Publication Date: April 10, 2020
Research article

A Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products

Maria T. Baltazar,[1] Sophie Cable, Paul L. Carmichael, Richard Cubberley, Tom Cull, Mona Delagrange, Matthew P. Dent, Sarah Hatherell, Jade Houghton, Predrag Kukic, Hequn Li, Mi-Young Lee, Sophie Malcomber, Alistair M. Middleton, Thomas E. Moxon, Alexis V. Nathanail, Beate Nicol, Ruth Pendlington, Georgia Reynolds, Joe Reynolds, Andrew White, and Carl Westmoreland

Unilever Safety and Environmental Assurance Centre, Colworth Science Park, Sharnbrook, Bedfordshire MK44 1LQ, UK

[1]To whom correspondence should be addressed. Fax: +44(0)1234 264 744. E-mail: maria.baltazar@unilever.com.



OECD
Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2021)35

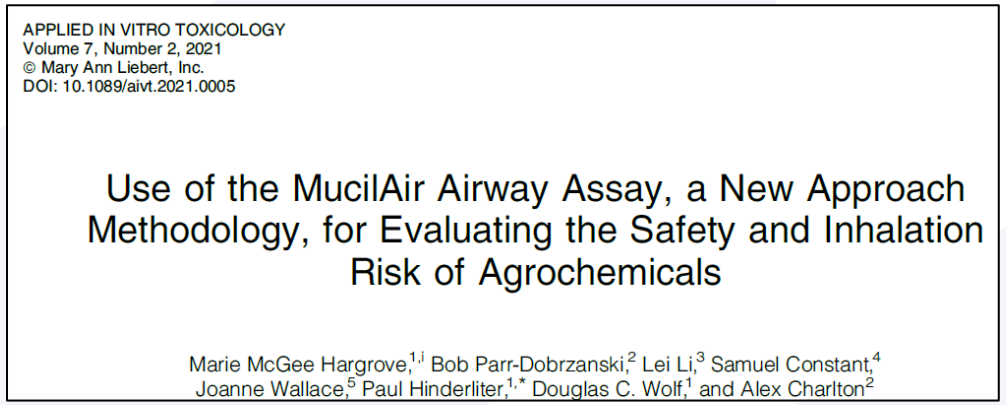
Unclassified — English - Or. English
27 October 2021

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

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

NAMs applied in real-life chemical safety assessments



APPLIED IN VITRO TOXICOLOGY
Volume 7, Number 2, 2021
© Mary Ann Liebert, Inc.
DOI: 10.1089/aivt.2021.0005

Use of the MucilAir Airway Assay, a New Approach Methodology, for Evaluating the Safety and Inhalation Risk of Agrochemicals

Marie McGee Hargrove,[1,i] Bob Parr-Dobrzanski,[2] Lei Li,[3] Samuel Constant,[4] Joanne Wallace,[5] Paul Hinderliter,[1,*] Douglas C. Wolf,[1] and Alex Charlton[2]

https://www.regulations.gov/document/EPA-HQ-OPP-2011-0840-0080

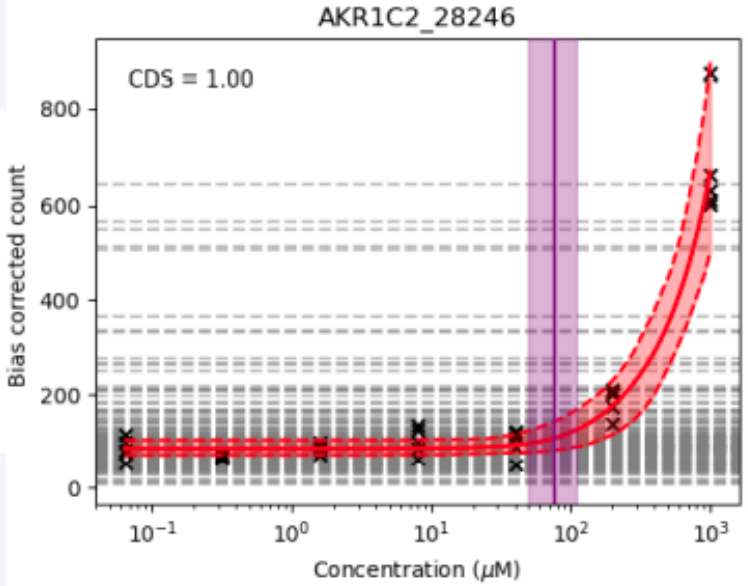
Unilever

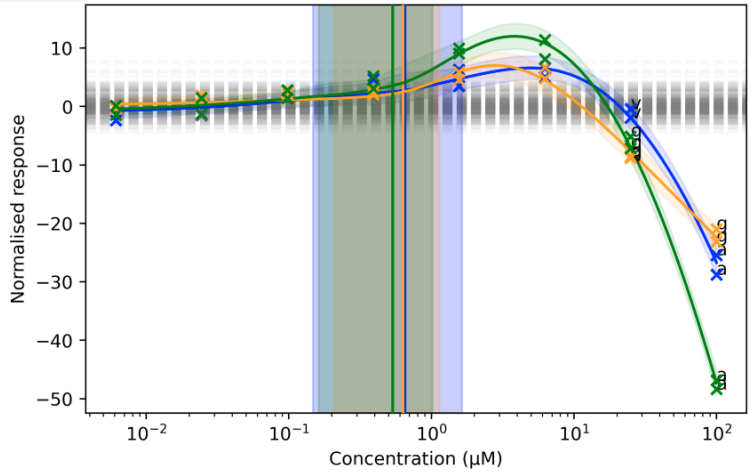
Stage 1: defining the benchmark chemical exposure scenarios

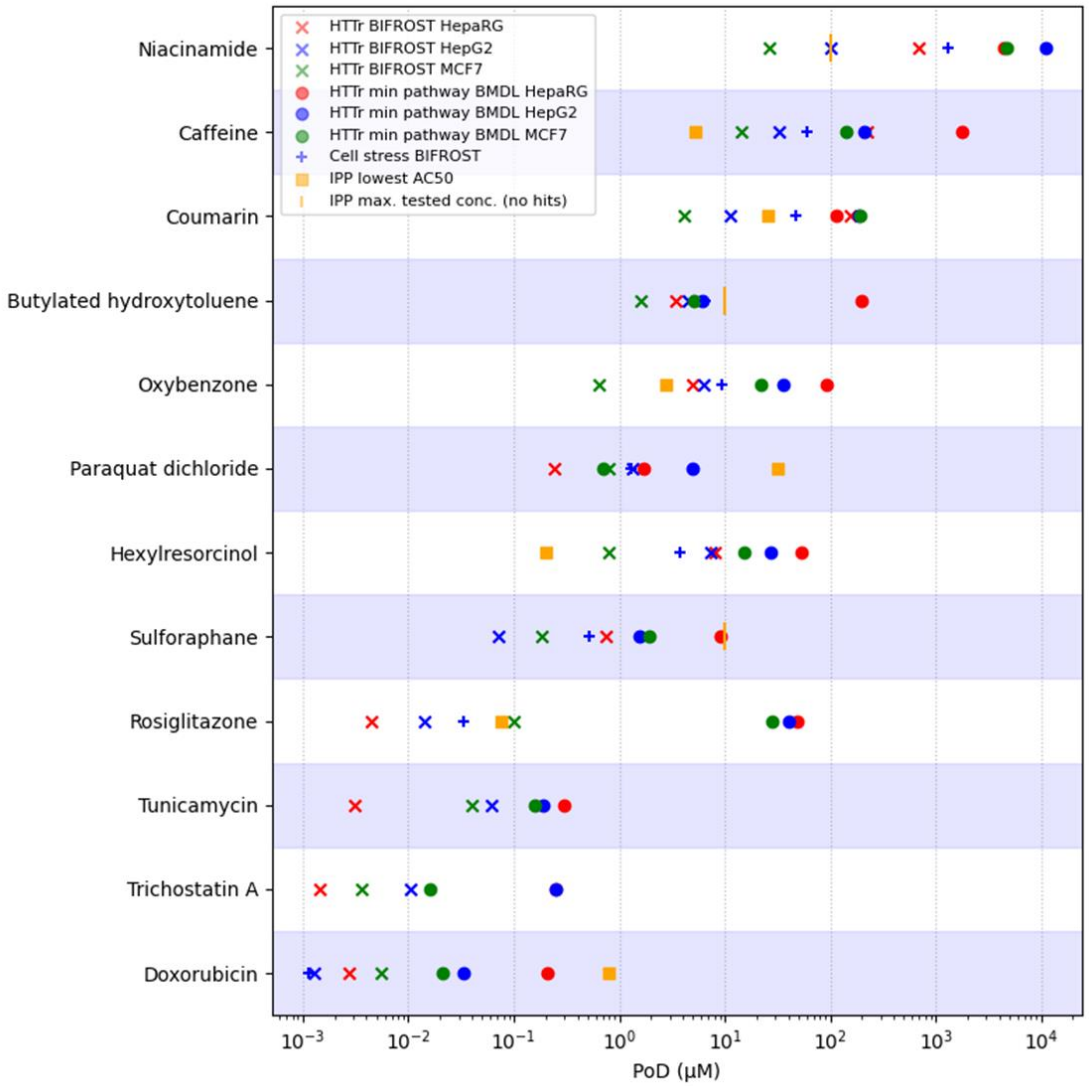
Chemical	Exposure scenario	Risk classification
Oxybenzone	**2 scenarios:** 0.5%; 2% sunscreen	Low risk
Caffeine	**2 scenarios:** 0.2% shampoo & coffee oral consumption 50 mg	Low risk
Caffeine	10g – fatal case reports	High risk
Coumarin	**3 scenarios:** 4 mg/d oral consumption; 1.6% body lotion (dermal); TDI 0.1 mg/kg oral	Low risk
Hexylresorcinol	**3 scenarios:** Food residues (3.3 ug/kg); 0.4% face cream; throat lozenge 2.4 mg	Low risk
BHT	Body lotion 0.5%	Low risk
Sulforaphane	**2 scenarios:** Tablet 60 mg/day; food 4.1-9.2 mg/day	Low risk
Niacinamide	**4 scenarios:** oral 12.5-22 mg/kg; dermal 3% body lotion and 0.1 % hair condition	Low risk
Doxorubicin	75 mg/m2 IV bolus 10 min; 21 days cycles; 8 cycles	High risk
Rosiglitazone	8 mg oral tablet	High risk
Valproic Acid (VPA)	**2 scenarios:** oral tablet 1000 mg & > 60 mg/kg	High risk
Paraquat	Accidental ingestion 35 mg/kg	High risk

Unilever

Stage 2: Estimating PODs from the different bioactivity assays

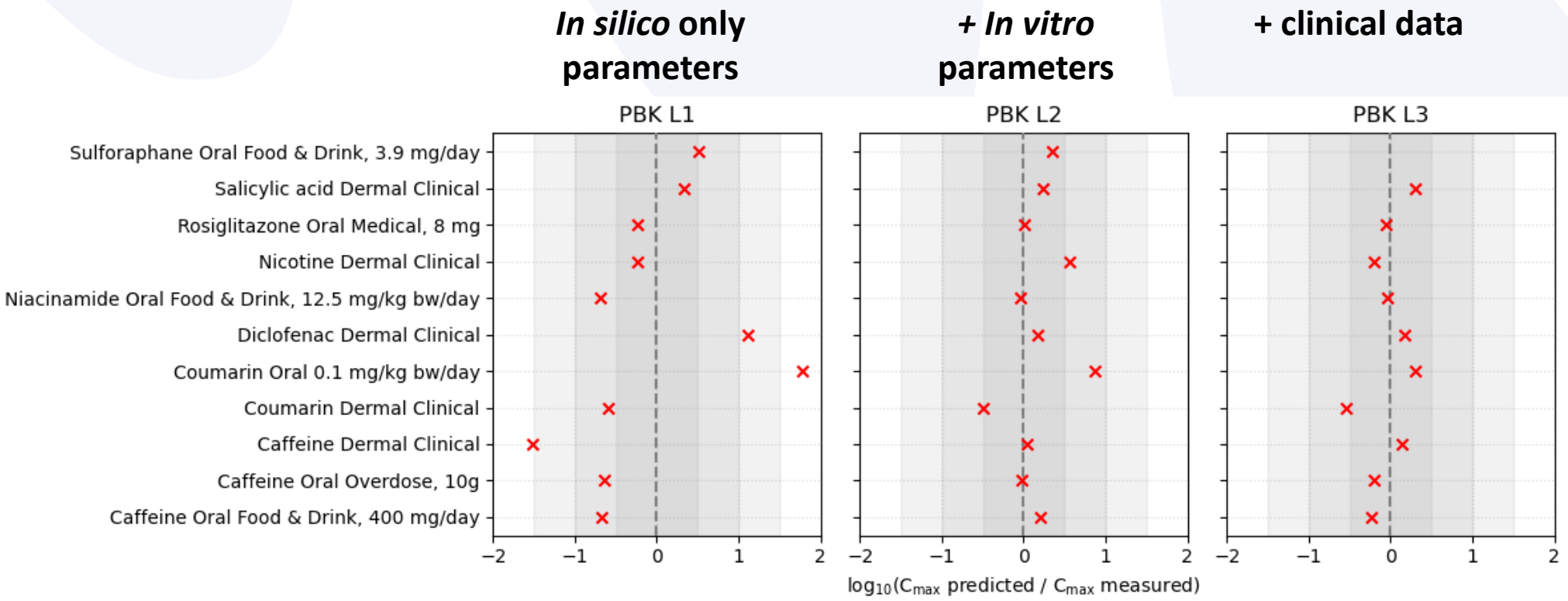






HTTr: High-throughput transcriptomics **CSP:** Cell Stress Panel **IPP:** In vitro pharmacological profiling

Considering the error in PBK models based on parameterisation level



- The PBK prediction error decreases as we go ‘up’ parameterisation levels
- Developed a Bayesian statistical model to quantify the error for a novel chemical

Unilever