Evaluation of a New Approach Methodology Toolbox for the Risk Assessment of Systemic Toxicity

Sophie Cable

Safety and Environmental Assurance Centre, Unilever

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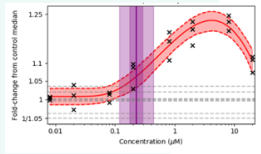
The overall goal is human safety assessment: exposure-led and human relevant

Potential hazards of the ingredients

Point of departure derived from concentration-response data

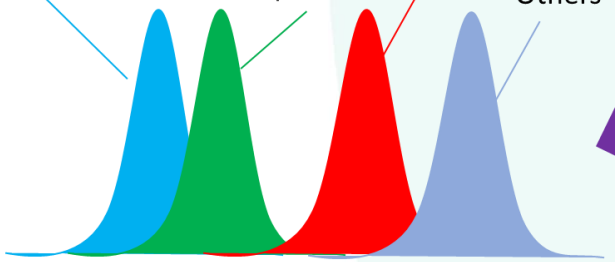


Cellular stress assays

Transcriptomics

Receptor binding

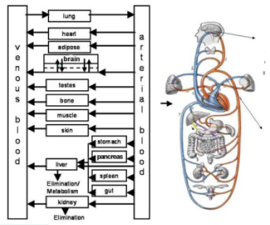
Others



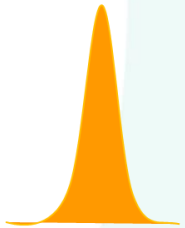
Consumer Exposure

Skin pen

Exposure models (PBK, free/total concentration)



Exposure estimation: Plasma C_{max}



Risk Assessment

Calculation of Bioactivity Exposure Ratio

The MoS is defined as the ratio the PoD and the relevant plasma C_{max} estimate

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A Case Study Approach to using NAMs in safety decision making.

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

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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.

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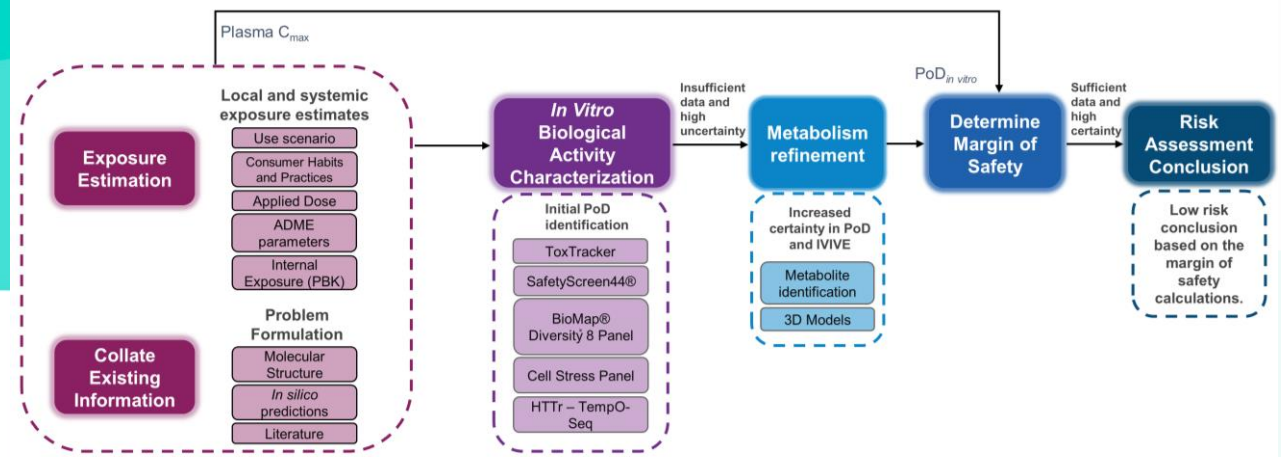
Application of physiologically based kinetic (PBK) modelling in the next generation risk assessment of dermally applied consumer products

Thomas E. Moxon*, Hequn Li*, Mi-Young Lee, Przemyslaw Piechota, Beate Nicol, Juliette Pickles, Ruth Pendlington, Ian Sorrell, Maria Teresa Baltazar

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



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Tox Spotlight article

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Research article

Identifying and Characterizing Stress Pathways of Concern for Consumer Safety in Next-Generation Risk Assessment

Sarah Hatherell,* Maria T. Baltazar,* Joe Reynolds,* Paul L. Carmichael,* Matthew Dent,* Hequn Li,* Stephanie Ryder,† Andrew White,* Paul Walker ,† and Alistair M. Middleton*,[1]

*Unilever Safety and Environmental Assurance Centre, Colworth Science Park, Sharnbrook, Bedfordshire MK44 1LQ, UK; and †Cyprotex Discovery Ltd, Macclesfield, Cheshire SK10 4TG, UK

Sarah Hatherell, Maria T. Baltazar, and Joe Reynolds contributed equally to this study.
[1]To whom correspondence should be addressed. Fax: +44(0)1234 264 744. E-mail: alistair.middleton@unilever.com.

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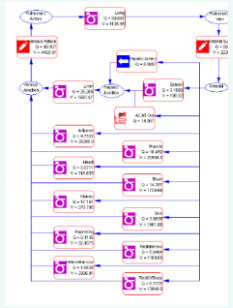
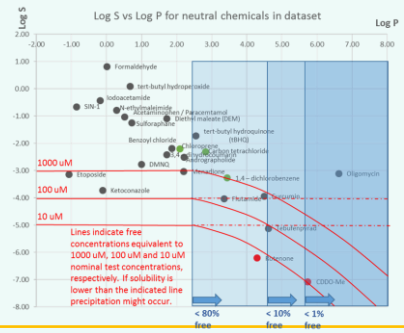
Overview of core toolbox

Exposure Estimation

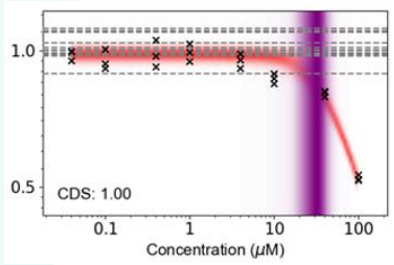
PBK models



Free concentration

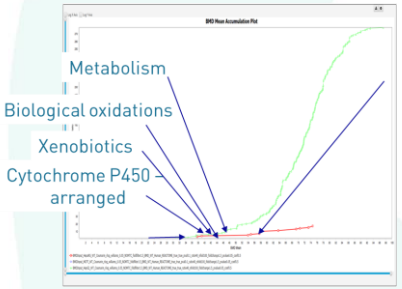


Concentration Response Models



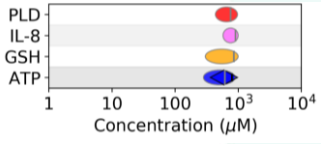
In Vitro Biological Activity Characterization

HTTr



- MCF7
- HepG2
- HepaRG

CSP

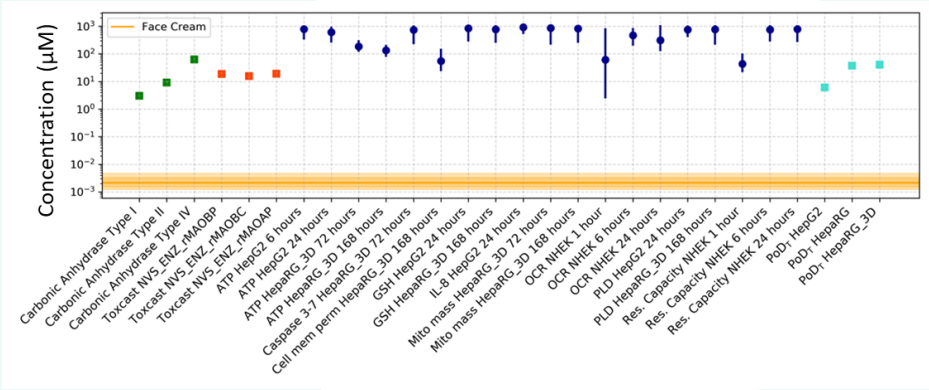


- HepG2

IPP

- All binding and enzymatic assay results were **negative at 10 μM, including COX-1 and COX-2**.
- Highest inhibition (22%) was for MAO-A

Margin of Safety estimate



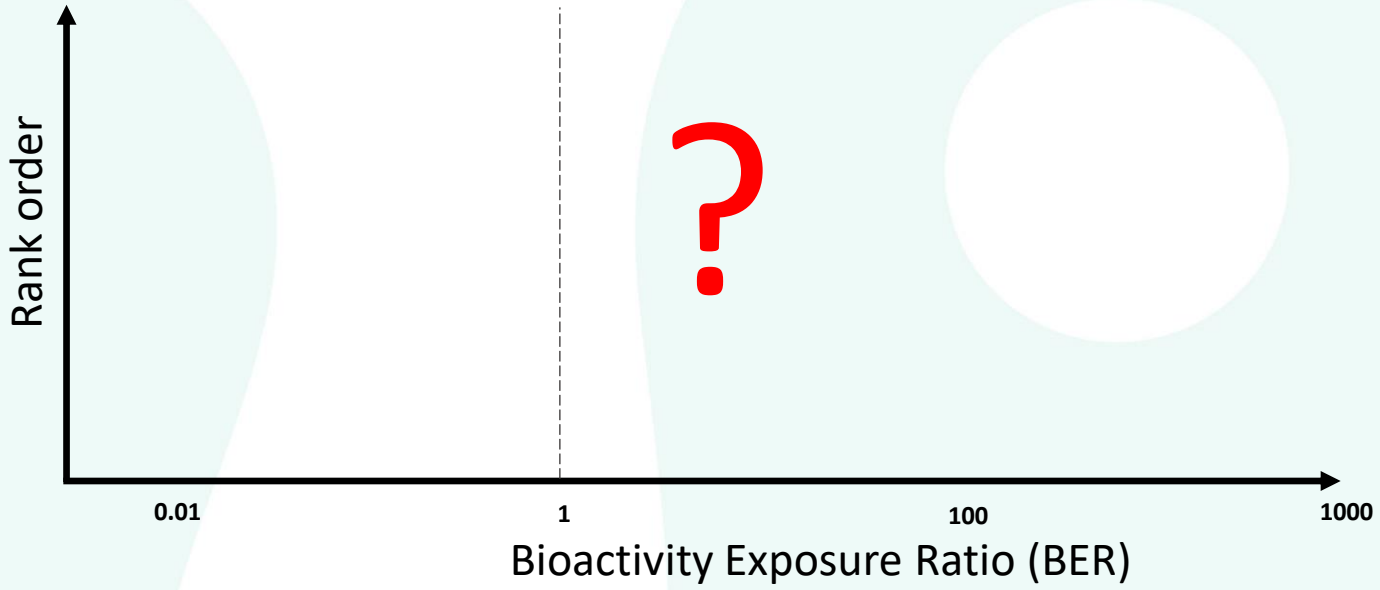
Inform safety decision

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

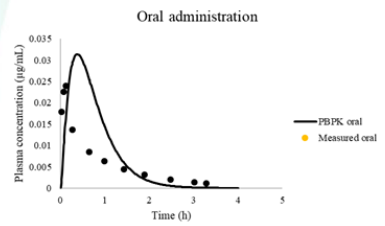
Evaluating the toolbox for risk assessment: a data driven approach

Chemical exposures scenarios

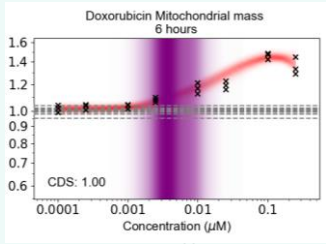
- ‘Low’ risk (from consumer goods perspective) – e.g. foods, cosmetics
- ‘High’ risk (from consumer goods perspective) – e.g. drugs



Define typical use-case scenarios benchmark chemical-exposures



PBK models of systemic exposure



In-vitro cell assays, estimate PoDs

Calculate the bioactivity exposure ration

Can the toolset successfully **distinguish between low and high risk** chemical exposure scenarios up to a certain BER?

Evaluating the toolset for risk assessment: a data driven approach

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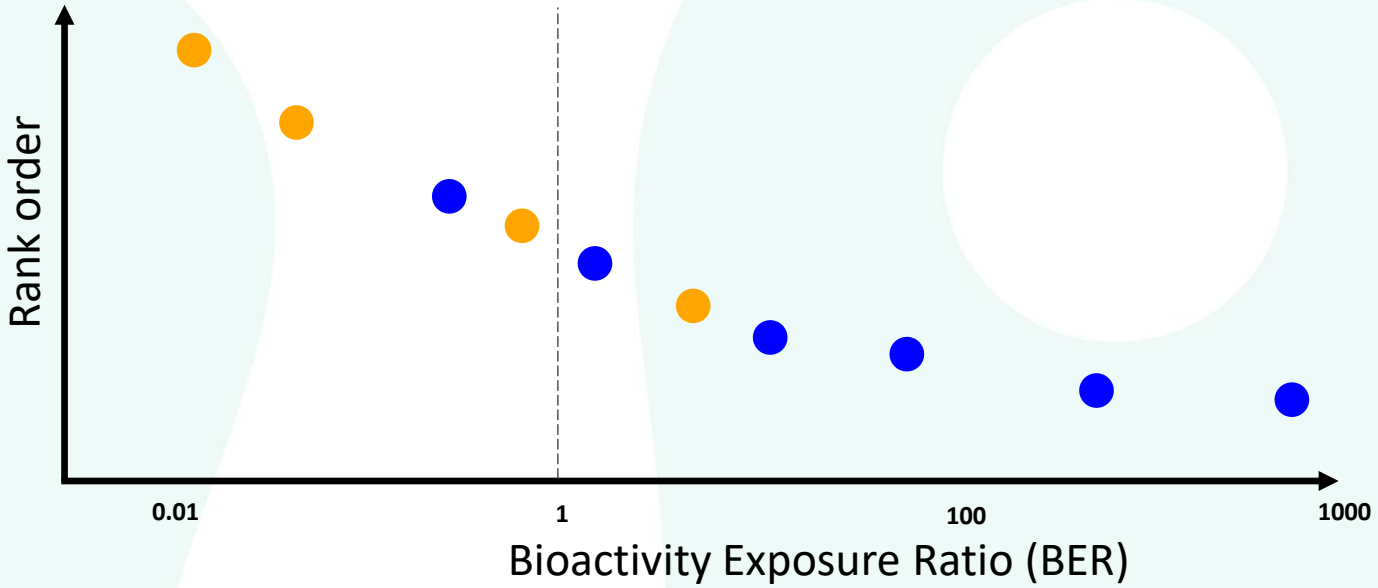

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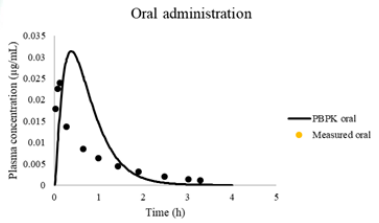

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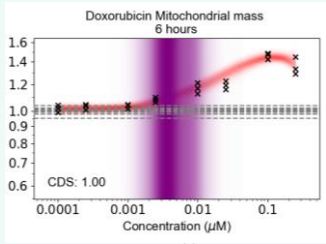


In-vitro cell assays, estimate PoDs

Calculate the bioactivity exposure ratio

Can the toolset successfully **distinguish between low and high risk** chemical exposure scenarios up to a certain BER?

Proof of concept testing with a subset of 12 chemicals.

Tiered approach to PBK Modelling

LEVEL 0
Estimate consumer exposure from use scenario

LEVEL 1
Using in silico parameters

LEVEL 2a
Using in vitro parameters

LEVEL 2b
Using in vitro parameters and human PK calibration

Adapted from Moxon et al, 2021

In Vitro Bioactivity Assays

Cellular Stress Panel

High content imaging assay in HepG2 cells with 24h treatment time. ~40 biomarkers measured across 10 stress pathways.

- Increased number of biological replicates
- Increased number of controls on the plate

High Throughput Transcriptomics (HTTr)

Temp-O-Seq technology with data generated in MCF7, HepG2 and 2D HepaRG cells with 24h treatment time.

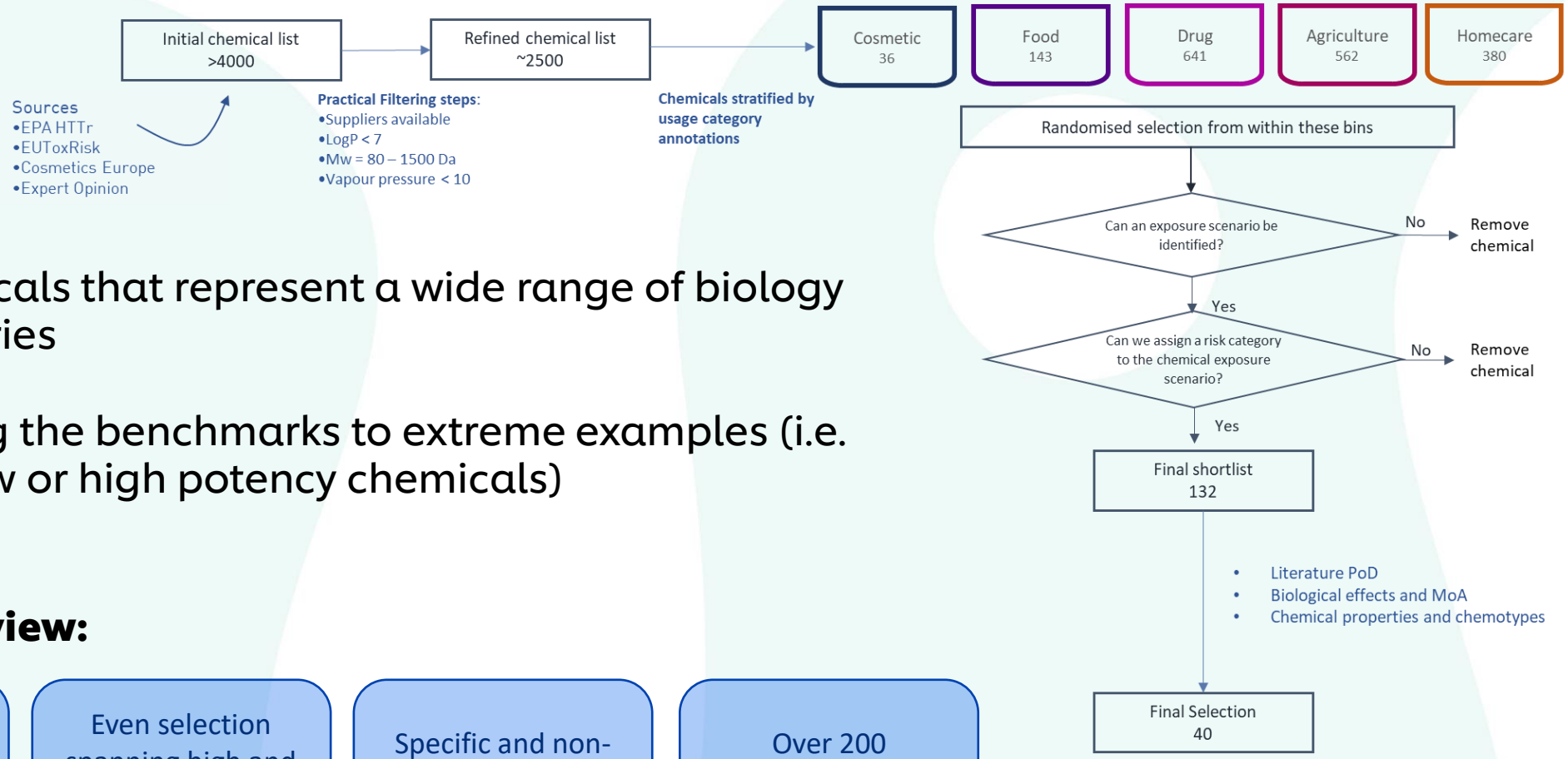
- Increased number of biological replicates
- Increased number of controls on plate – DMSO and positive control compounds
- Altered plate layout

***In Vitro* Pharmacological Profiling**

Binding, enzymatic, coactivator recruitment and luciferase assays of pharmacologically relevant targets.

- Increased panel of targets from 44 to 63 to include more nuclear receptors amongst others.
- Change in screening concentration depending on cytotoxicity.

Benchmark chemical selection



Aim:

- Select chemicals that represent a wide range of biology and chemistries
- Avoid biasing the benchmarks to extreme examples (i.e. extremely low or high potency chemicals)

Selection overview:

At least 4 compounds with multiple exposure scenarios

Even selection spanning high and low risk chemical-exposure scenarios

Specific and non-specific modes of action identified.

Over 200 chemotypes covered

Neurotoxicity

Hepatotoxicity

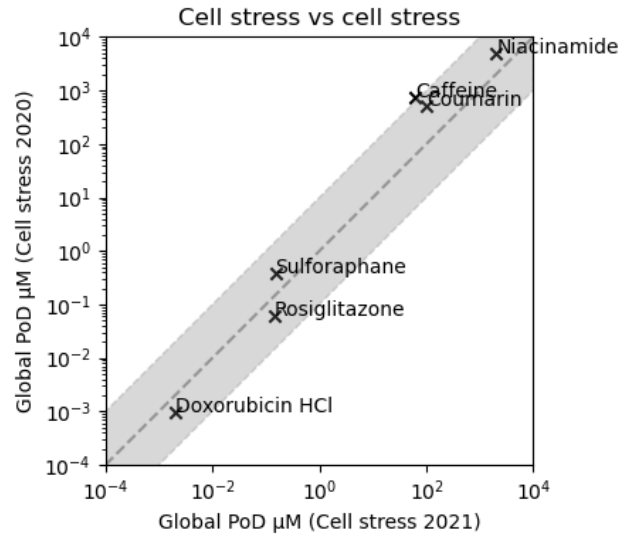
Mitochondrial toxicity

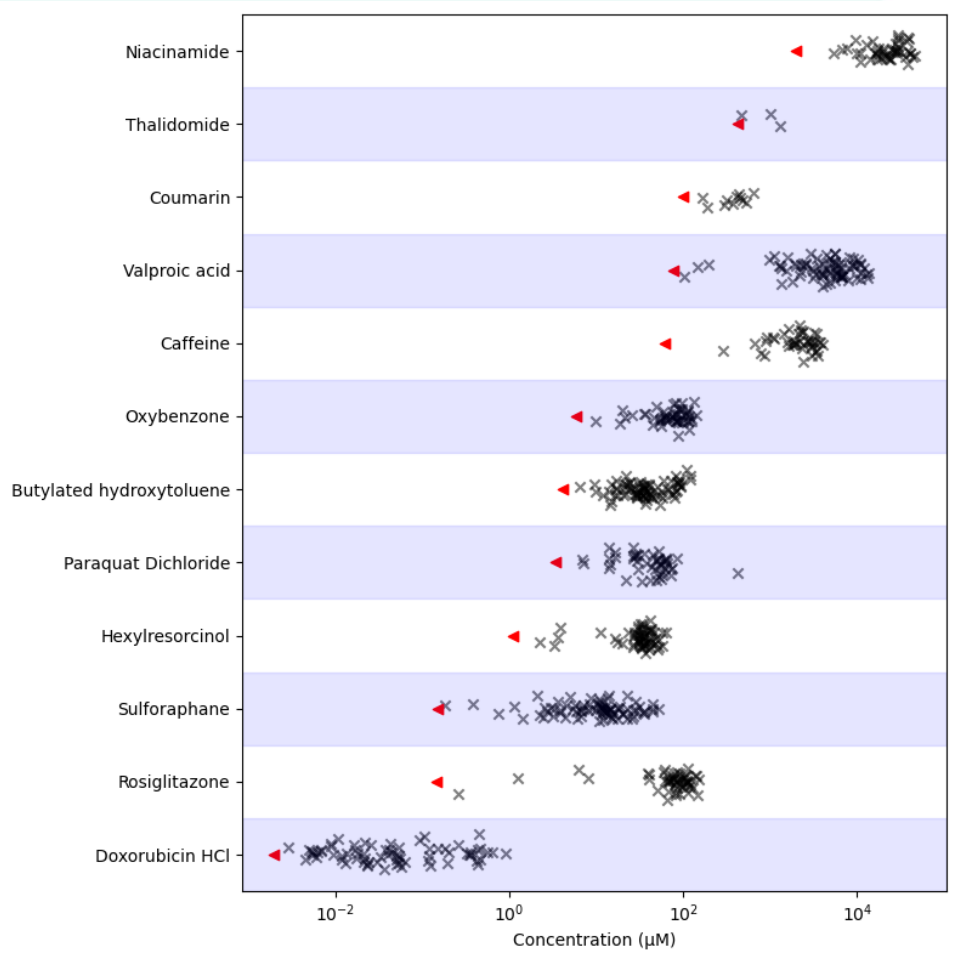
Methaemaglobinema

Examples of adverse biological effects evidenced in the literature for the final selection chemicals

Preliminary cellular stress results for an initial sub-selection of compounds

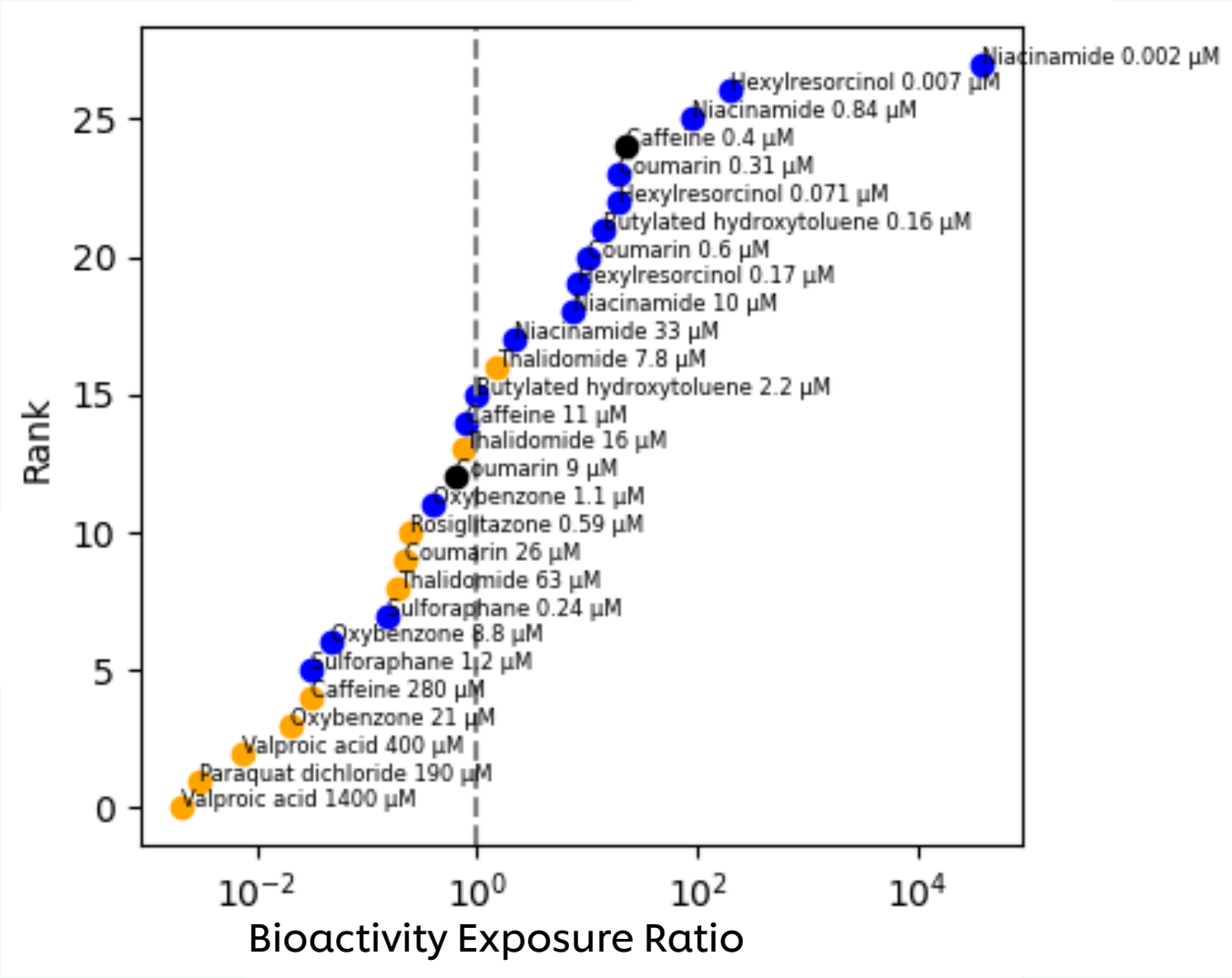
- Early stages looked at reproducibility of assays and impact of optimisation steps
- Initial list of 12 chemicals: 6 replicas and 6 new ones.
- Data analysis ongoing for cell stress, transcriptomics and pharmacological profiling.
- Encouraging PoD reproducibility across the cell stress experiments.





Preliminary summary of cell stress points of departure across all biomarkers and biological replicates

Calculation of the BER for multiple exposure scenarios using preliminary data from proof of concept testing



Cmax estimates were calculated using in silico parameters only (i.e. Level 1 PBK Model).

The minimum point of departure from the cell stress panel assays and the HTTr was used to calculate the BER.

'Low' risk (from consumer goods perspective) – e.g. foods, cosmetics

'High' risk (from consumer goods perspective) – e.g. drugs

Concluding remarks

- Case studies have demonstrated it is possible to integrate exposure estimates and bioactivity points of departure to make a safety decision.

- 'Early tier' in vitro screening tools show promise for use in a protective rather than predictive capacity.

- Evaluation of NGRA needs to be in the context of how to combine (often many different) estimates of exposure and bioactivity to give reproducible decisions on safety with transparent measurement of uncertainty

- Large scale evaluation exercises can increase confidence in using mathematical tools to define a *protective* BER.

- Through the process of this evaluation we can identify gaps in our approaches and design new testing strategies to address them. E.g. where can more advanced tools such as microphysiological systems be useful in NGRA?

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Acknowledgements

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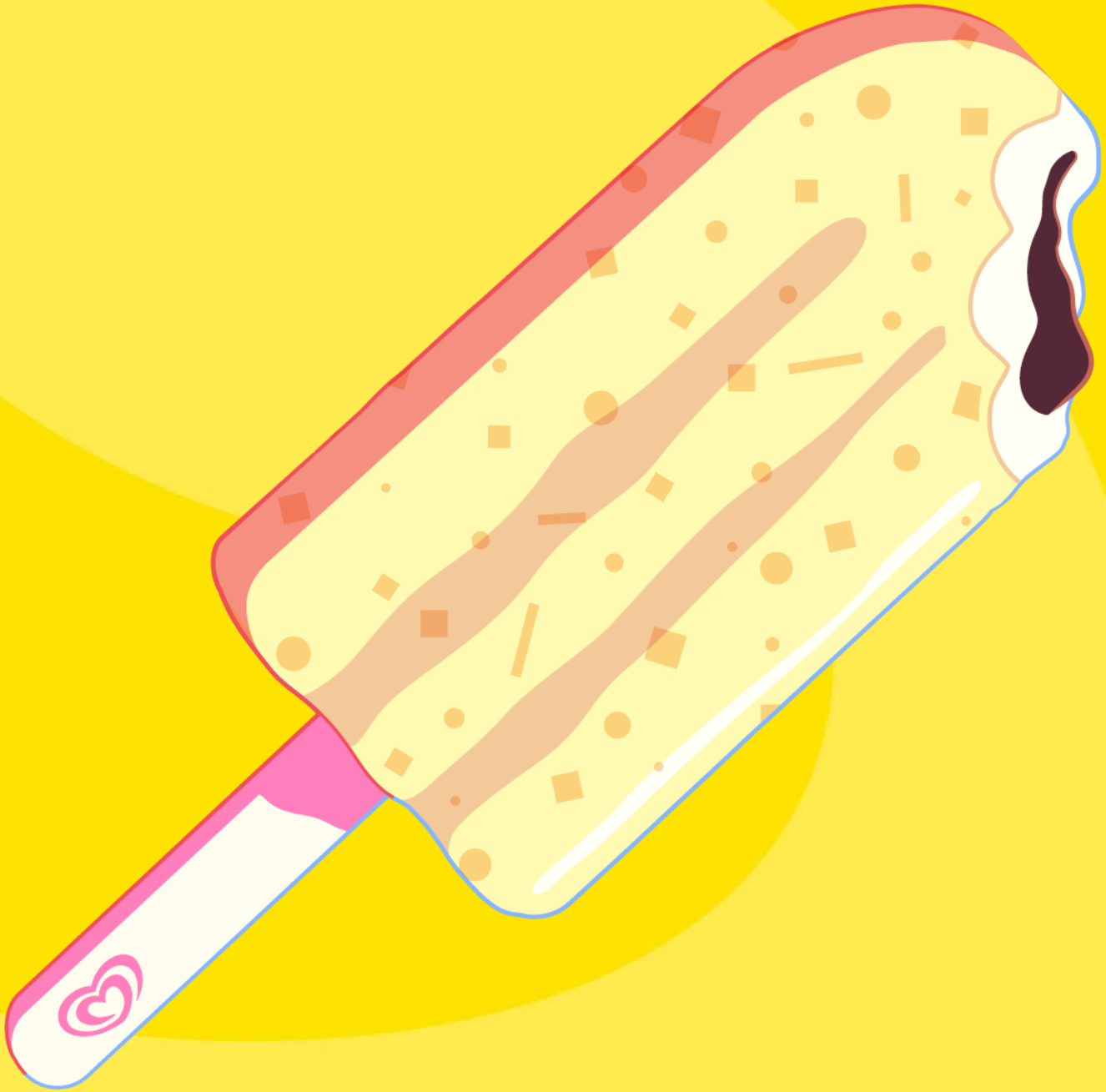

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