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Building Confidence in Non-animal Safety Frameworks for Systemic Toxicity

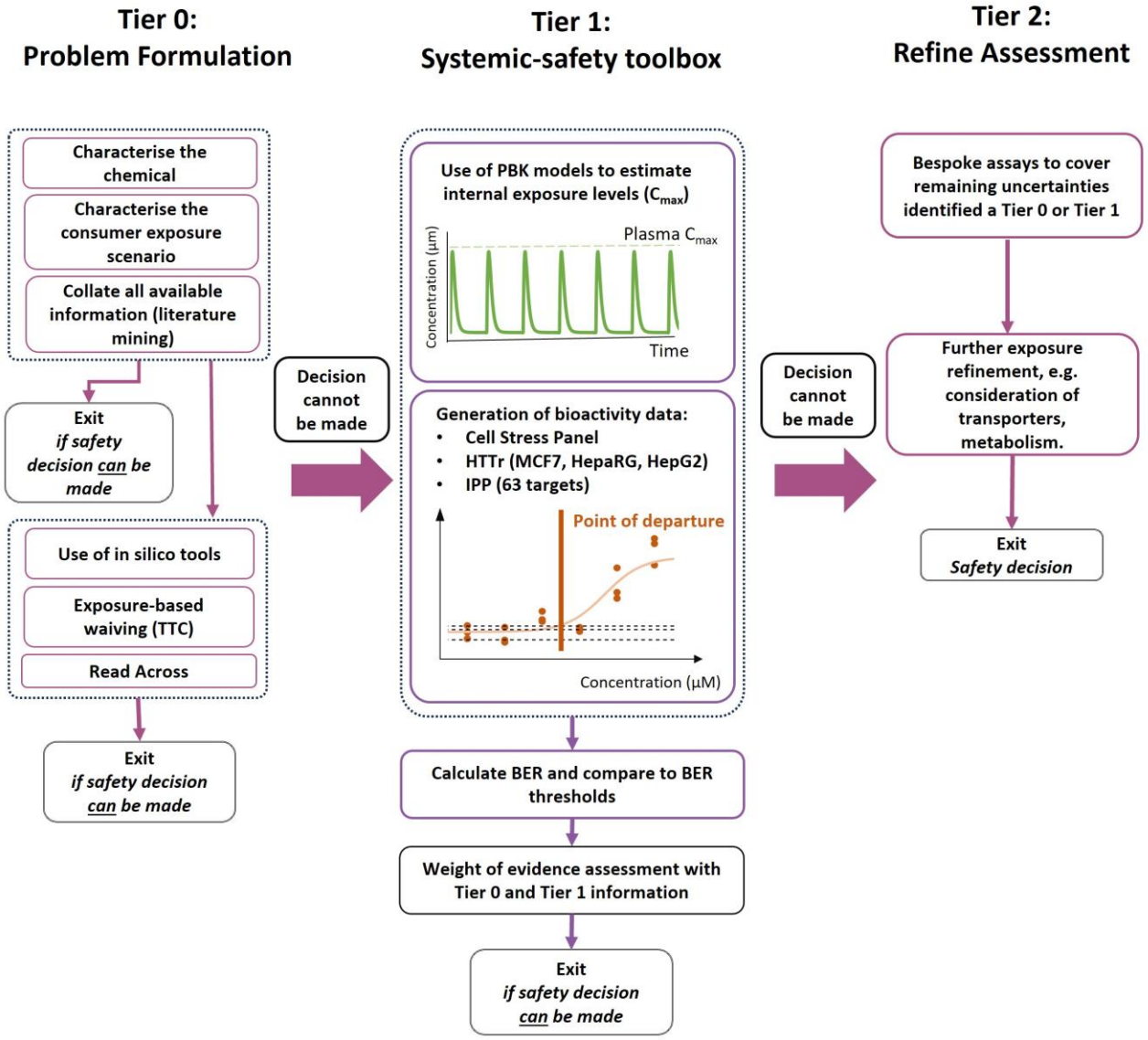
Predrag Kukic

July 1st 2026

SERS
Safety, Environmental & Regulatory Science

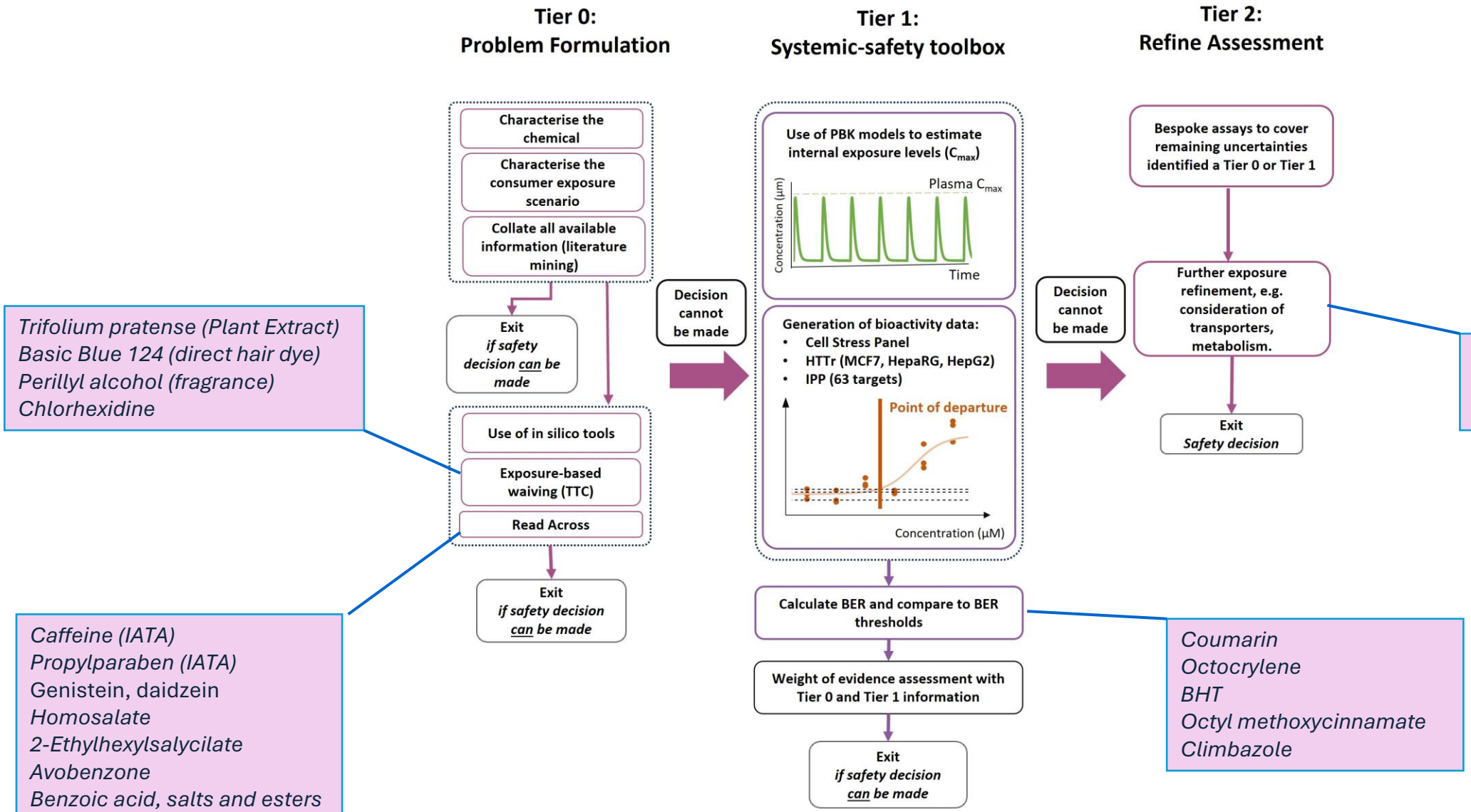



Focus: Repeated Dose Systemic Toxicity



Baltazar et al., (2025). https://pubmed.ncbi.nlm.nih.gov/40396742/

Focus: Repeated Dose Systemic Toxicity



Trifolium pratense (Plant Extract)
Basic Blue 124 (direct hair dye)
Perillyl alcohol (fragrance)
Chlorhexidine

Caffeine (IATA)
Propylparaben (IATA)
Genistein, daidzein
Homosalate
2-Ethylhexylsalycilate
Avobenzone
Benzoic acid, salts and esters

Phenoxyethanol (IATA)
Benzophenone 4
Butyl benzyl salicylate

Coumarin
Octocrylene
BHT
Octyl methoxycinnamate
Climbazole

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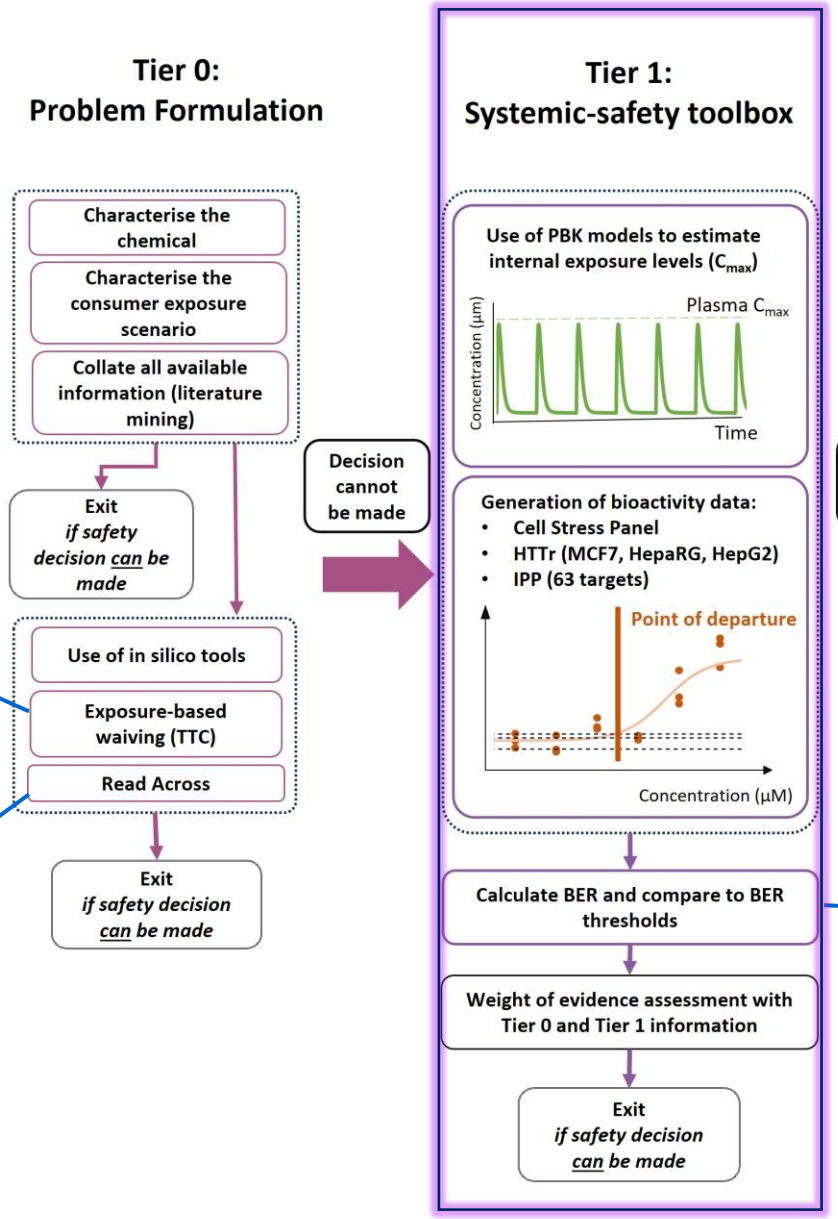

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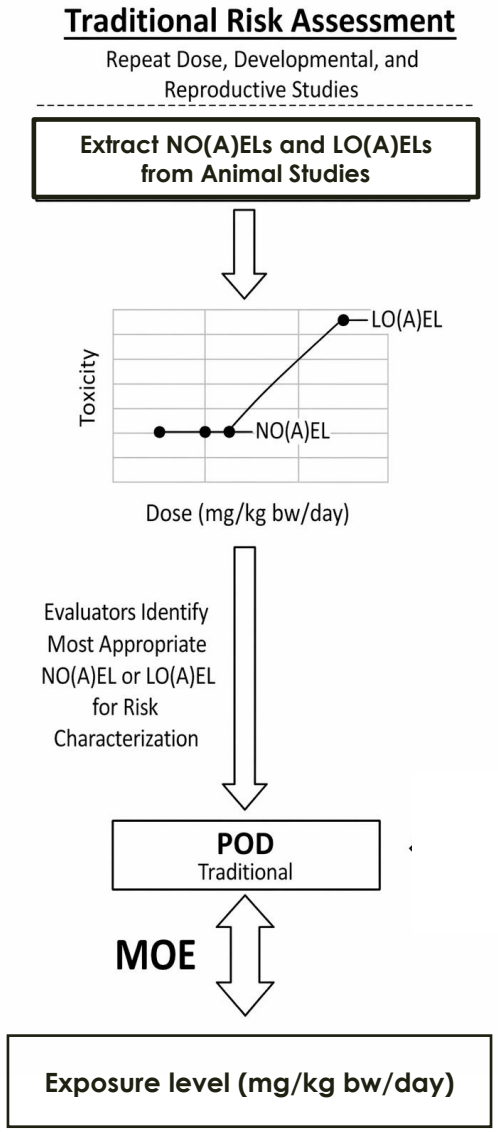
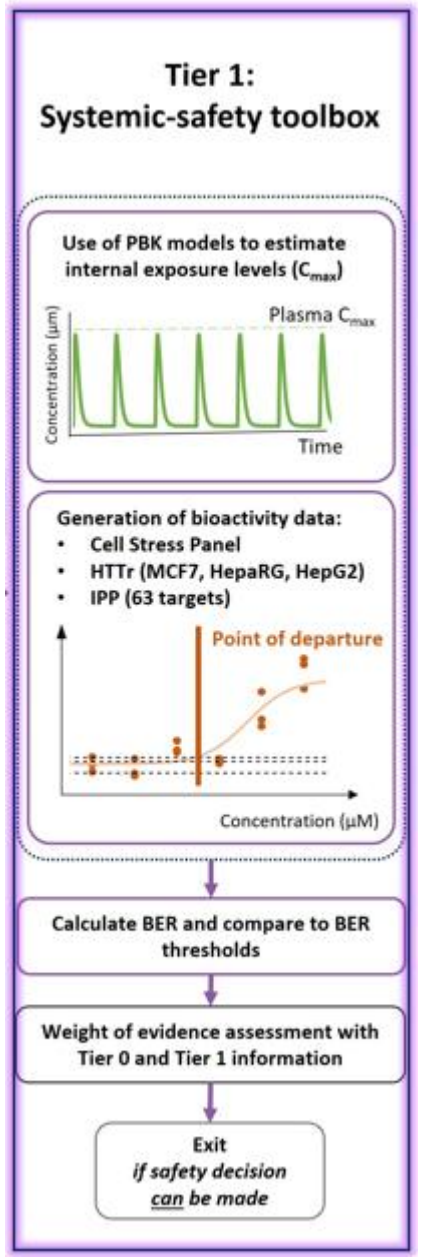
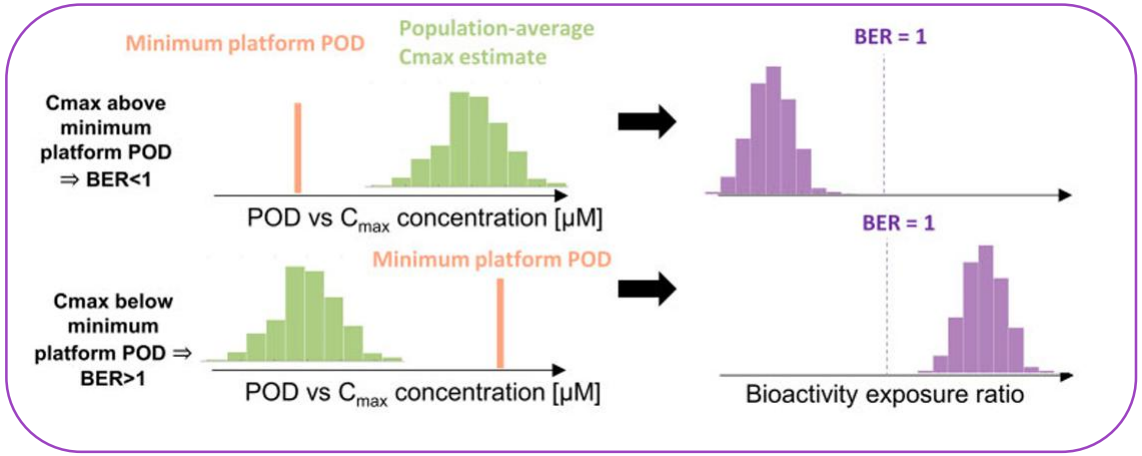
Systemic-safety Toolbox



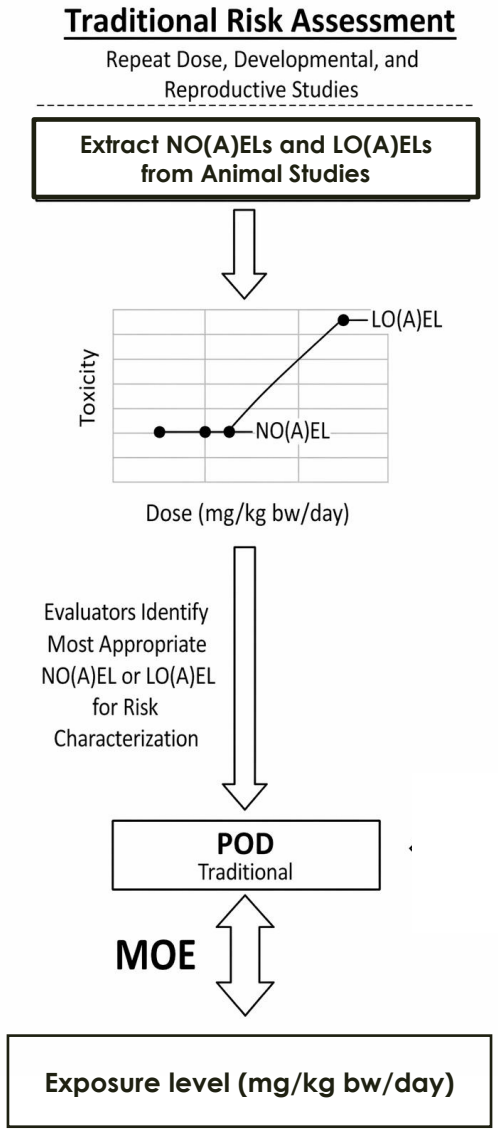
Adapted from Health Canada





Middleton et al., 2022. https://doi.org/10.1093/toxsci/kfac068

Systemic-safety Toolbox - CoU



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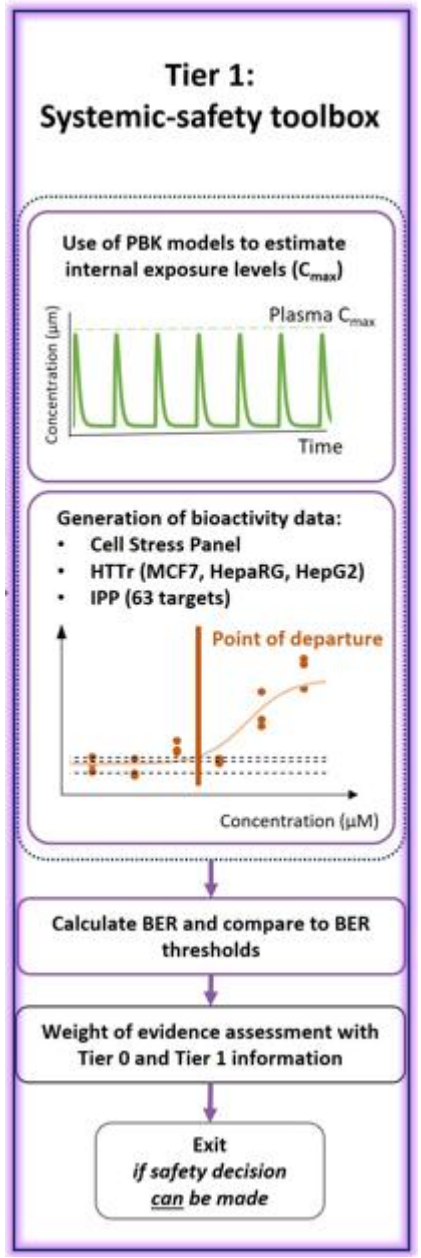

Context of Use (Systemic-safety Toolbox)

- **NAM-based toolbox** intended to be used as **a Tier 1 within a NGRA/IATA framework** for systemic toxicity (i.e. quantitative risk assessment of ingredients in **consumer goods products**).
- Provides **protective thresholds (PoDs) for systemic toxicity**.
- Provides **better or equivalent levels of protection of human** health comparing to traditional animal studies and useful for risk assessment by deriving protective decision thresholds (BER)

Systemic-safety Toolbox – Evaluation Strategy

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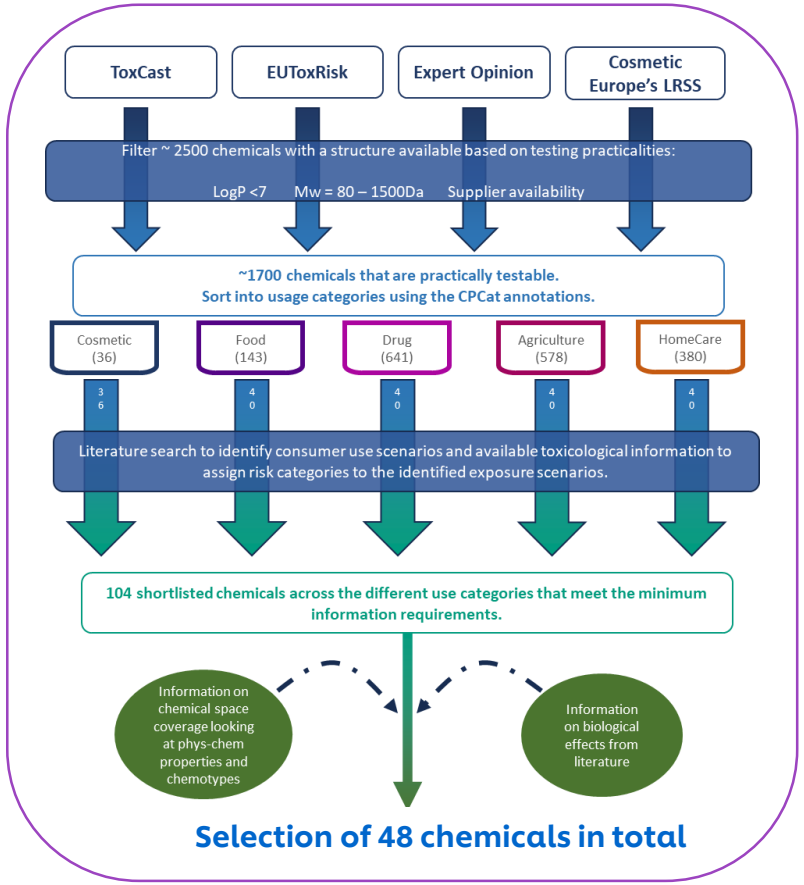
Select test chemicals

Maximise coverage of different **chemistries and biological effects/toxicity.**

Set performance criteria

1) The performance of the NAM toolbox is **assessed against historical safety decisions.**
2) How do *in vitro* PoDs compare to *in vivo* PoD?



Chemical	Exposure scenario	Risk classification
Oxybenzone	**2 scenarios:** 0.5%; 2% sunscreen	Low risk
Caffeine	**2 scenarios:** 0.2% shampoo & coffee oral consumption 400 mg/day	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
Paraquat	Accidental ingestion 35 mg/kg	High risk

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Cable S et al., (2024). https://doi.org/10.1093/toxsci/kfae159
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Systemic-safety Toolbox – Evaluation Strategy

Select test chemicals

Maximise coverage of different **chemistries and biological effects/toxicity.**

Set performance criteria

1) The performance of the NAM toolbox is **assessed against historical safety decisions.**
2) How do *in vitro* PoDs compare to *in vivo* PoD?

Define the toolbox components

Choose a set of NAMs covering exposure modelling and **bioactivity which provide wide biological coverage.**

Assess protectiveness of the toolbox based on BER

Assess protection level comparing to historical safety decisions/animal studies.

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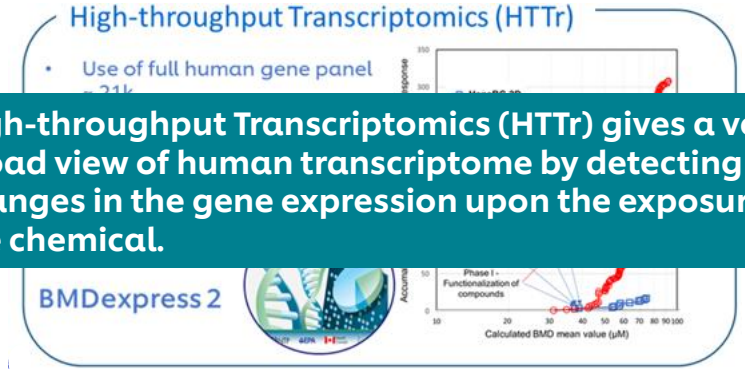
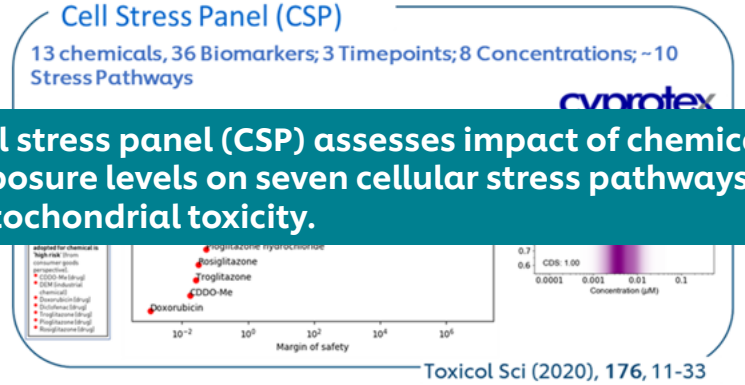
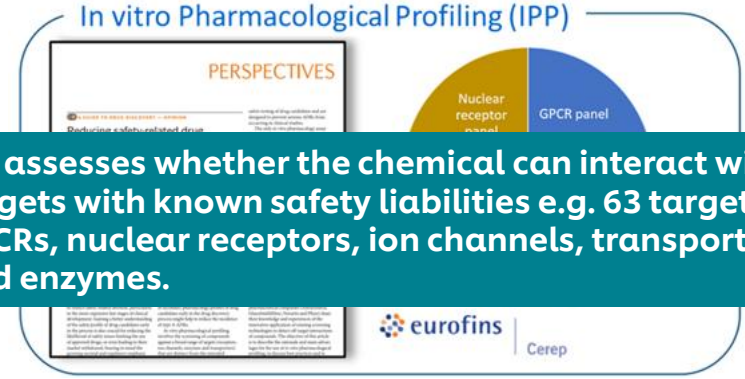
Systemic-safety Toolbox – NAM Components



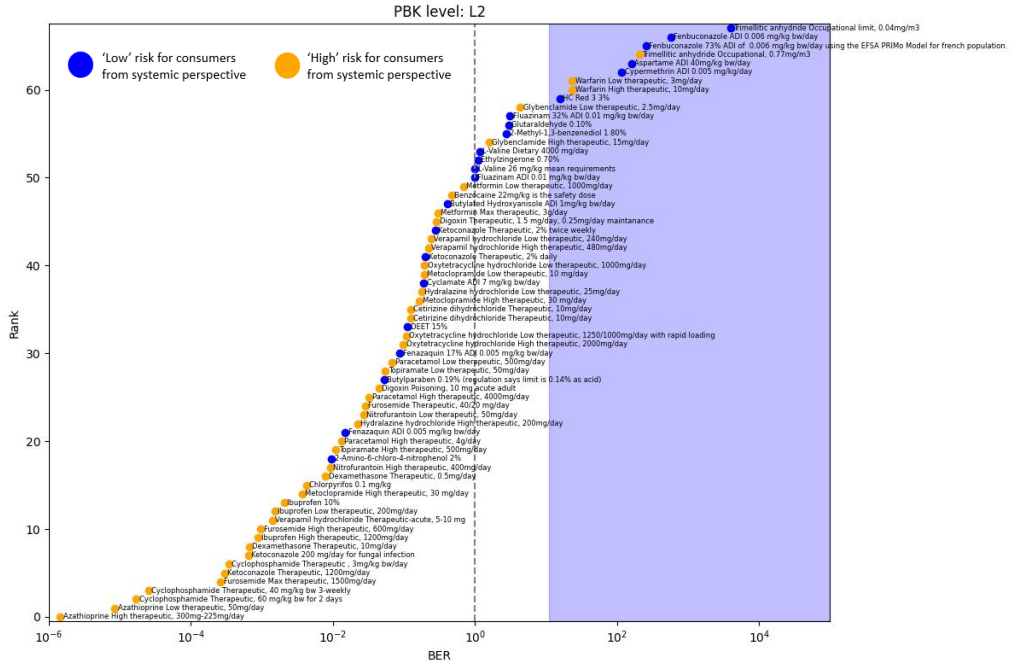
High-throughput Transcriptomics (HTTr) gives a very broad view of human transcriptome by detecting any changes in the gene expression upon the exposure to the chemical.



Cell stress panel (CSP) assesses impact of chemical exposure levels on seven cellular stress pathways and mitochondrial toxicity.



IPP assesses whether the chemical can interact with targets with known safety liabilities e.g. 63 targets: GPCRs, nuclear receptors, ion channels, transporters and enzymes.



- **Systemic Toolbox is protective for 43/46 of the high-risk exposure scenarios (96% protectiveness), BER < Threshold**
- **Systemic Toolbox identifies 6/22 of the low-risk exposure scenarios (27% utility), BER > Threshold**
- **Chemical- Exposure scenarios not protective for:**
  - Warfarin therapeutic oral dose (3mg/day & 10mg/day)
  - Trimellitic anhydride inhalation exposure

Cable S et al., (2024). https://doi.org/10.1093/toxsci/kfae159
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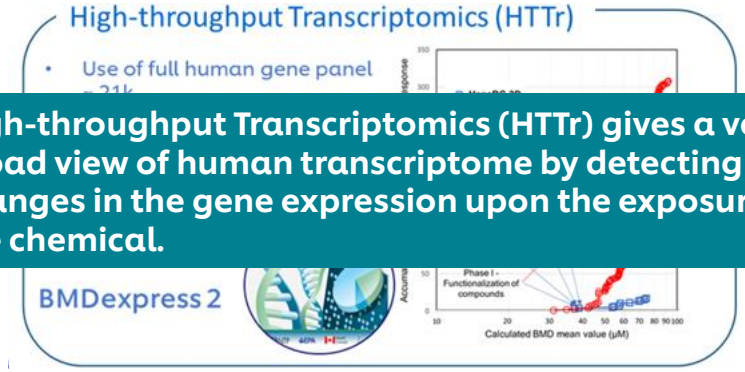
Systemic-safety Toolbox – Evaluation



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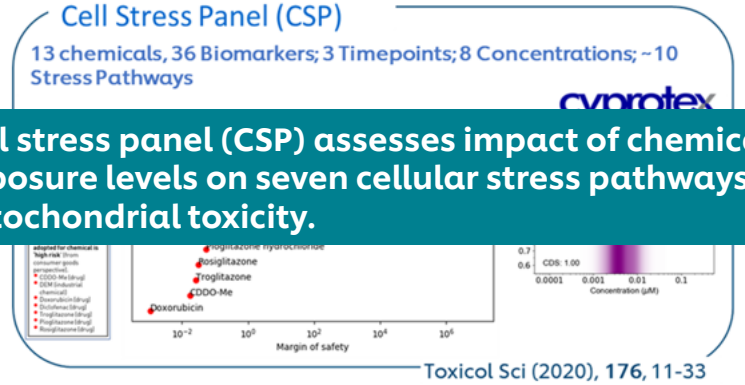

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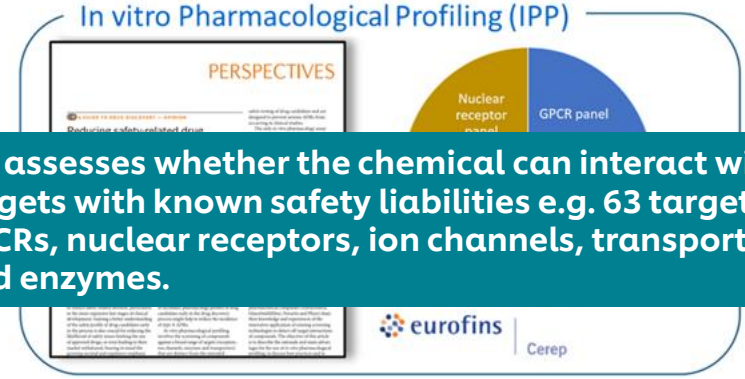

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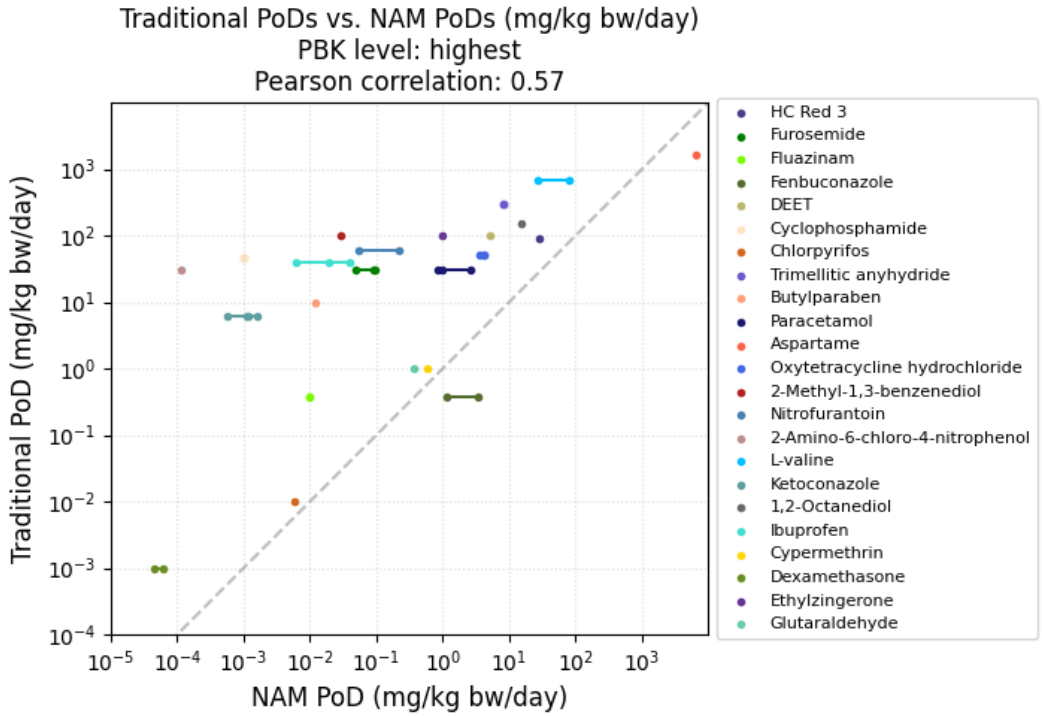


- For 25 overlapping chemicals the lowest *in vivo* NOAEL or NOEL was identified from three sources: ToxRefDB, the supplementary material of Paul-Friedman et al (2020) and published regulatory opinions.
- **Systemic Toolbox is more conservative (24/25 cases) than lowest *in vivo* NOAELs or NOELs**

Cable S et al., (2024). https://doi.org/10.1093/toxsci/kfae159
Middleton et al., 2022. https://doi.org/10.1093/toxsci/kfac068

Systemic-safety Toolbox – Alternative NAM Components 1

Unilever

Prof Ivan Rusyn

Article | Open access | Published: 08 November 2022

Developing an internal threshold of toxicological concern (iTTC)

Jon A. Arnot, Liisa Toose, James M. Armitage, Alessandro Sangion, Alexandra Looky, Trevor N. Brown, Li Li & Richard A. Becker

Journal of Exposure Science & Environmental Epidemiology **32**, 877–884 (2022) | Cite this article

11k Accesses | **20** Citations | **1** Altmetric | Metrics

Regulatory Toxicology and Pharmacology 114 (2020) 104656

Contents lists available at ScienceDirect

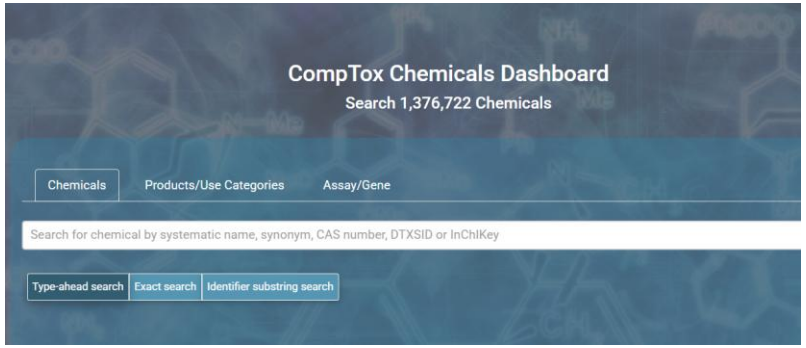
Regulatory Toxicology and Pharmacology

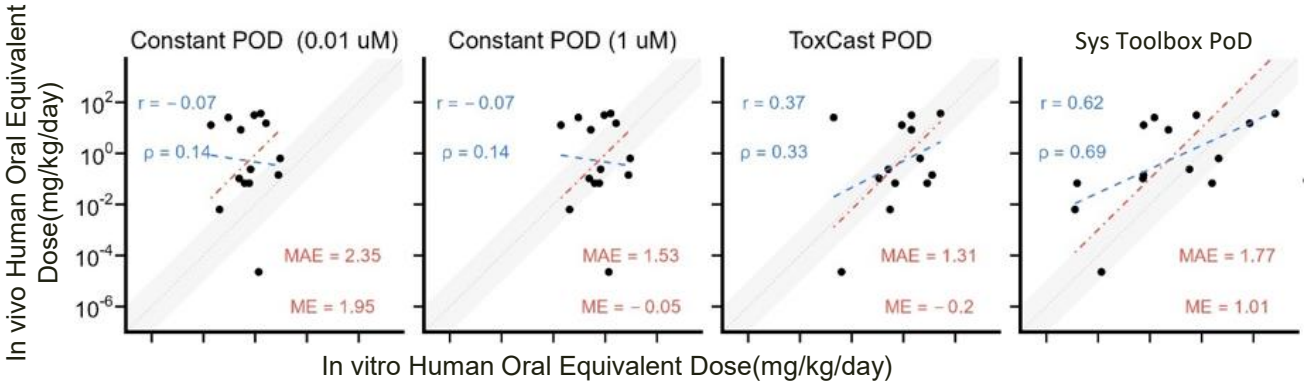
journal homepage: www.elsevier.com/locate/yrtph

An interim internal Threshold of Toxicologic Concern (iTTC) for chemicals in consumer products, with support from an automated assessment of ToxCast™ dose response data

Karen L. Blackburn, Gregory Carr, Jane L. Rose*, Bastian G. Selman

The Procter & Gamble Company, Mason Business Center, 8700 Mason Montgomery Road, Cincinnati, OH, 45040, United States





- Across the chemical data set, **cell assay-derived PODs** generally showed **higher correlation** coefficients (ρ and r) **compared to those using fixed iTTC values**.

- Concordance was generally **improved** when using **PODs from Sys Toolbox compared to ToxCast-based PODs** (5th percentile of all active AC50).

Hsing-Chieh Lin et al., (2025). https://www.sciencedirect.com/science/article/pii/S305062042500051X

Systemic-safety Toolbox – Alternative NAM Components 2

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EPA

Dr Josh Harrill lab

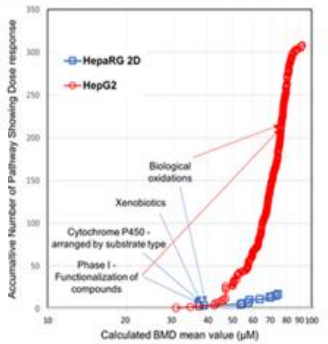
Cell Line	Description
ASC52Telo	• hTERT immortalized mesenchymal stem cell • Fibroblast-like morphology • Adipose tissue
CCD-18Co	• Colonocyte from a healthy donor • Fibroblast morphology • Colon
CHON-001	• hTERT immortalized chondrocyte cell • Fibroblast-like morphology • Long bone, cartilage
HBEC3-KT	• hTERT immortalized epithelial cell • Epithelial, packed cuboidal morphology • Lung, bronchus
HepG2	• Hepatocellular carcinoma cell • Epithelial-like morphology • Liver

Cell Line	Description
hNP1	• Derived from a neuroepithelial lineage of human embryonic stem cells • Neural progenitor cells
Ker-CT	• hTERT immortalized keratinocyte cells • Epithelial morphology • Skin, foreskin
RPTEC/TERT-1	• hTERT immortalized epithelial cell • Epithelial morphology • Kidney, proximal tubule
TeloHAEC	• hTERT immortalized cells • Endothelial morphology • Heart, aorta
U-2 OS	• Osteosarcoma cell • Epithelial morphology • Bone

High-throughput Transcriptomics (HTTr)

- Use of full human gene panel ~ 21k
- 24 hrs exposure
- 7 concentrations

- **6 hTERT**
- **2 cancer**
- **2 healthy donor cell lines**



High-throughput phenotypic profiling (HTPP)

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- **6 hTERT**
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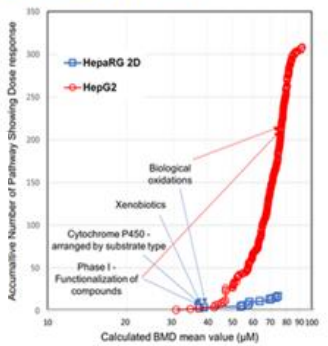

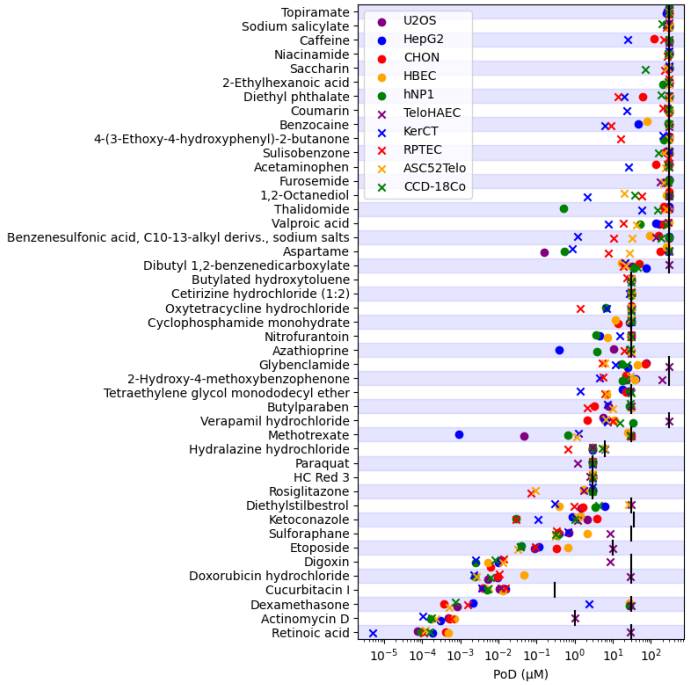

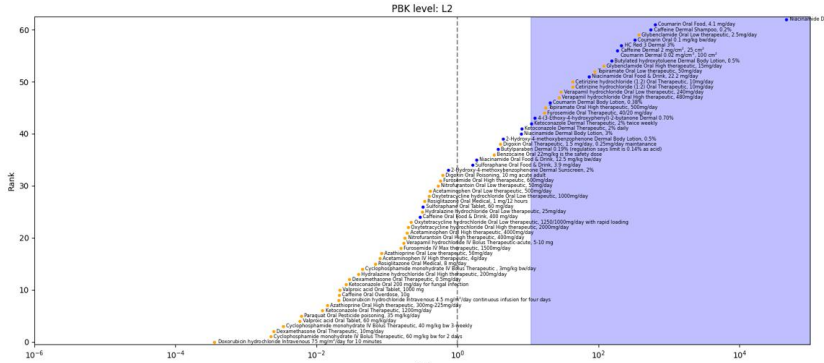

- Initial results using PoDs from HTTr in **10 cell lines do not show improved protectiveness and utility** of the Toolbox.
- Initial results point to the direction that the **IPP panel is necessary** for a high level of protectiveness.
- Work in progress to integrate HTTr and HTTP data in 10 cell lines and assess against the identical risk classifications.

Systemic-safety Toolbox – Alternative NAM Components 3

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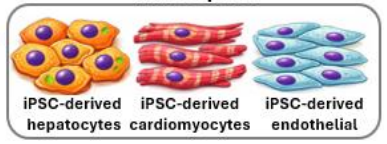
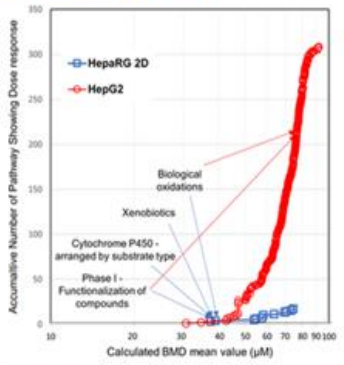
Prof Ivan Rusyn lab

High-throughput Transcriptomics (HTTr)

- Use of full human gene panel ~ 21k
- 24 hrs exposure
- 7 concentrations

TAMU's panel





High-throughput phenotypic profiling (HTPP)

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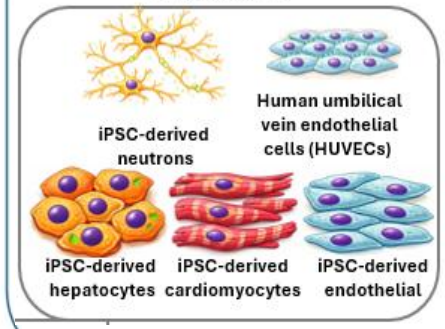

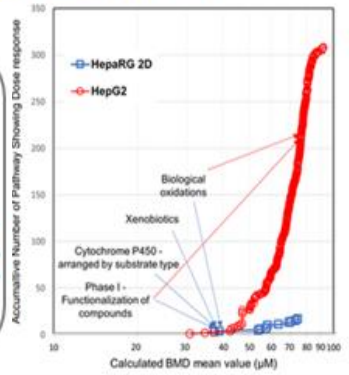

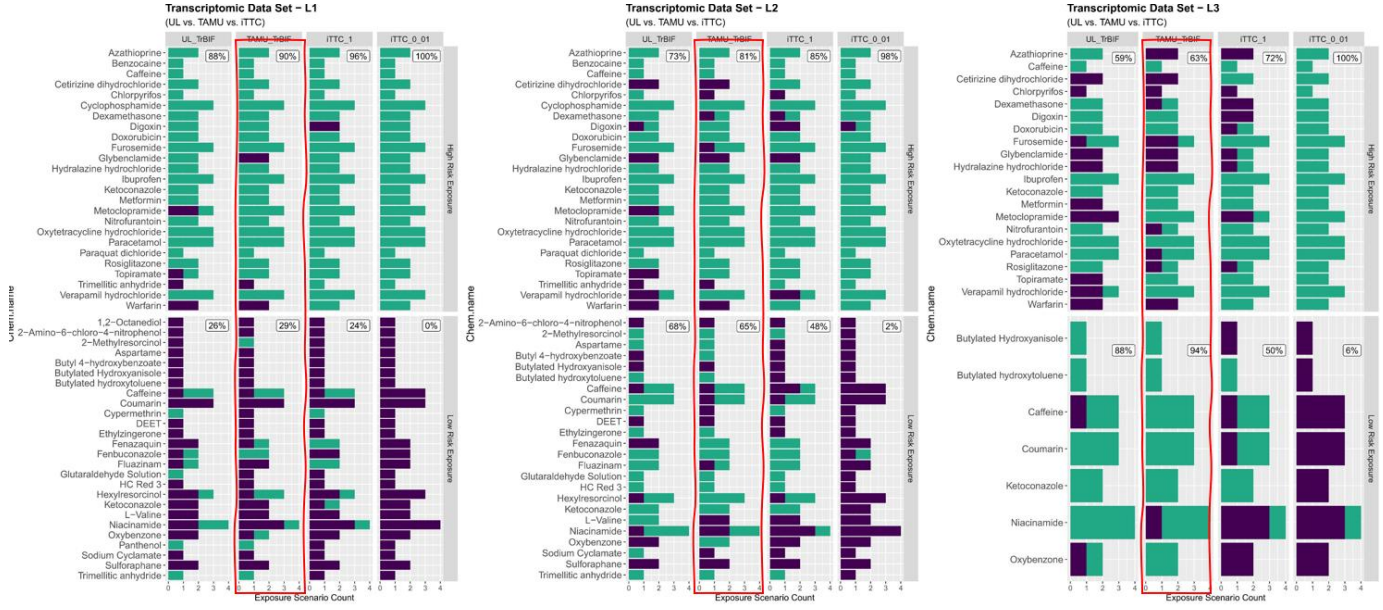

- Initial results using PoDs from HTTr in **3 iPSC cell lines and HTPP in 5 cell lines do not show improved protectiveness and utility** of the Toolbox.
- Work in progress to integrate HTTr and HTTP data assess against the identical risk classifications.

Conclusions and Next Steps

- To gain scientific confidence it is important:
  - To **clearly define CoU**: Systemic-safety Toolbox is intended to be used as a Tier 1 within the NGR A/IATA framework and should provide protective PoDs for systemic toxicity.
  - Perform **evaluation in a systematic way**
- There are many different combinations of NAMs that can make up a Systemic-safety Toolbox
- Optimisation of the Systemic-safety Toolbox is desirable and can be approached by comparing protection and utility of different NAM combinations using identical exposure scenarios and risk classification metrics.
- Next Steps:
  - Optimisation of the Systemic-safety Toolbox
  - Detailed characterisation of uncertainties and variations of the NAM components

Acknowledgements

Unilever

Dr Josh Harrill lab Prof Ivan Rusyn lab

40+ years of developing non-animal safety science

70+ collaborations

600+ publications

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