Evaluating a systemic safety toolbox for use in Next Generation Risk Assessment

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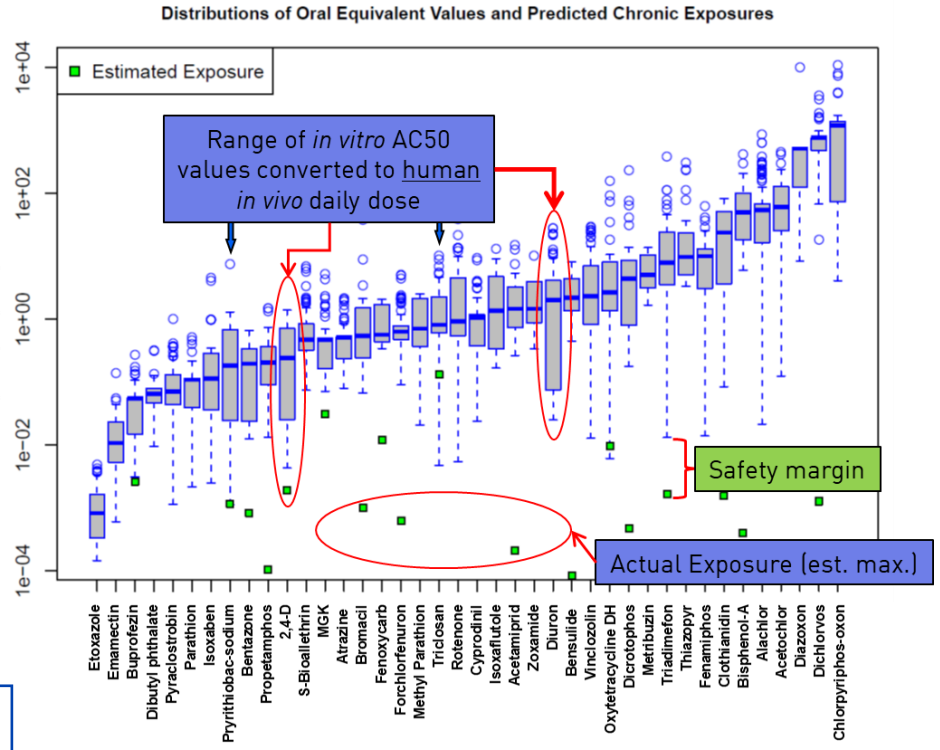
Middleton et al (2022), *Tox Sci,* Volume 189, Issue 1, Pages 124-147

Safety without animal testing - Next Generation Risk Assessment (NGRA)

*NGRA is defined as **an exposure-led, hypothesis-driven** risk assessment approach that **integrates New Approach Methodologies (NAMs)** to assure **safety without the use of animal testing***

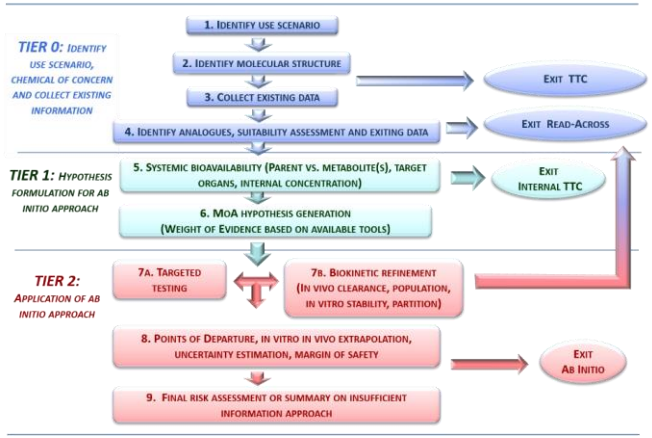


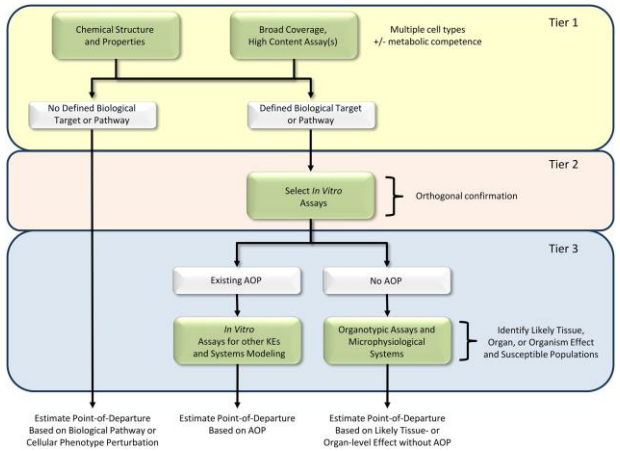
Slide from Dr Rusty Thomas, EPA, with thanks

Rotroff, *et al.* Tox.Sci 2010

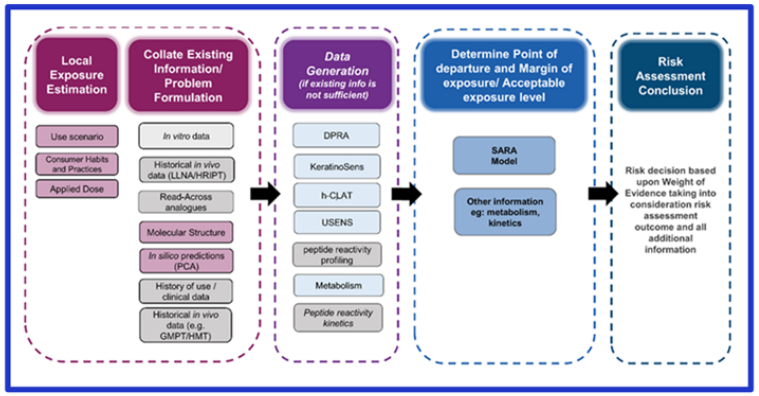
The hypothesis underpinning this type of NGRA is that **if there is no bioactivity observed at consumer-relevant concentrations, there can be no adverse health effects.**

Decision frameworks in NGRA



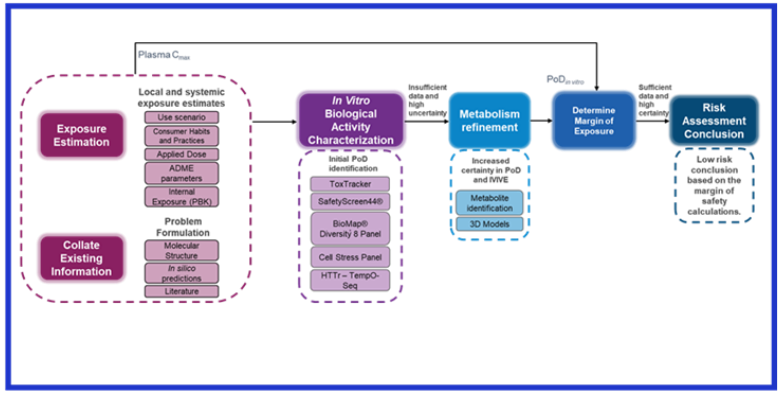


Skin Sensitisation



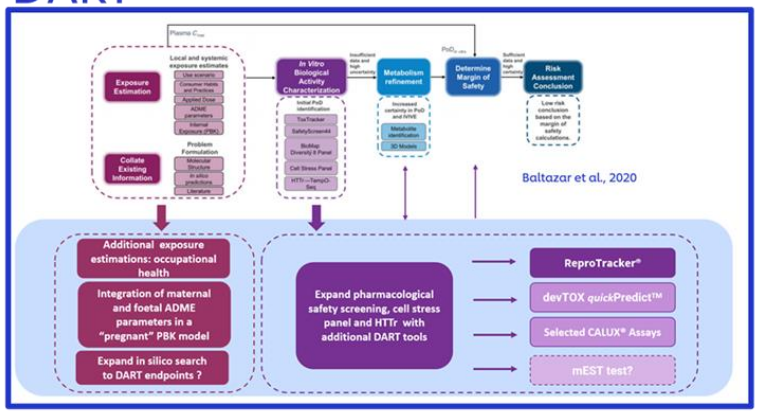
Reynolds et al (2021) Reg Tox Pharmacol, **127**, 105075

Systemic safety



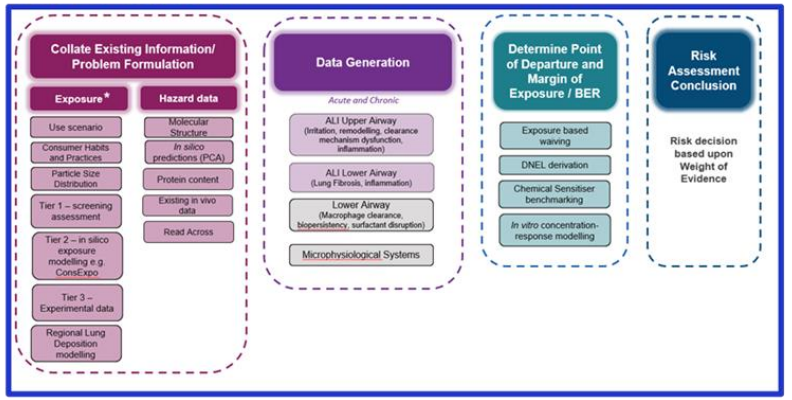
Baltazar *et al.*, (2020) *Tox Sci* , Volume 176, Issue 1, Pages 236–252

DART



Rajagopal et al (2022). Front. Toxicol., 07 March 2022

Inhalation



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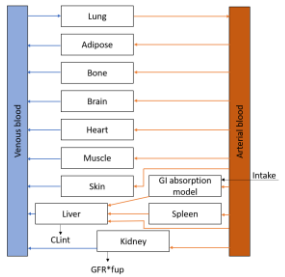
Decision frameworks in NGRA

Problem formulation – Tier 0

Initial BER estimate – Tier 1

Use case

Exposure route

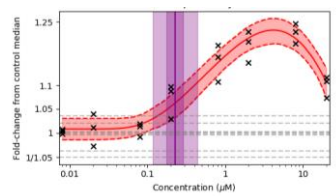


Exposure

Assay 1

Assay 2

Assay 3



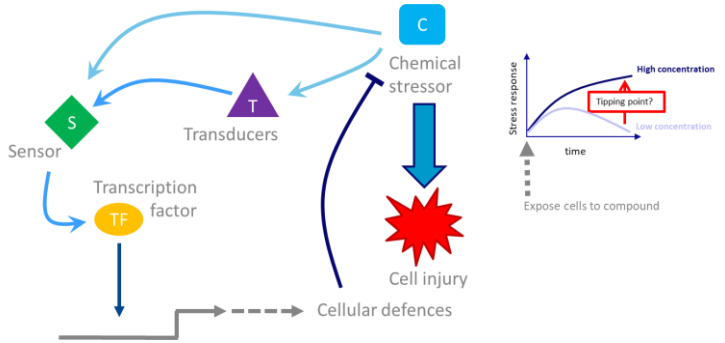
Hazard

- Internal exposure levels estimated using PBK models
- Set of assays covering broad range of biological effects & PODs.
- Key output: Bioactivity Exposure Ratio (**BER=POD/Exposure**)

Uncertain risk: exposure may trigger bioactivity

Small BER (i.e., Exposure close to POD)

BER refinement – Tier 2



- Refinement of internal exposure estimates:
  - Using clinical data
- Refined understanding of biological effects:
  - Repeat dose dynamics
  - Distinguishing bioactivity and adversity
  - Microphysiological systems

Low risk of exposure causing any bioactivity

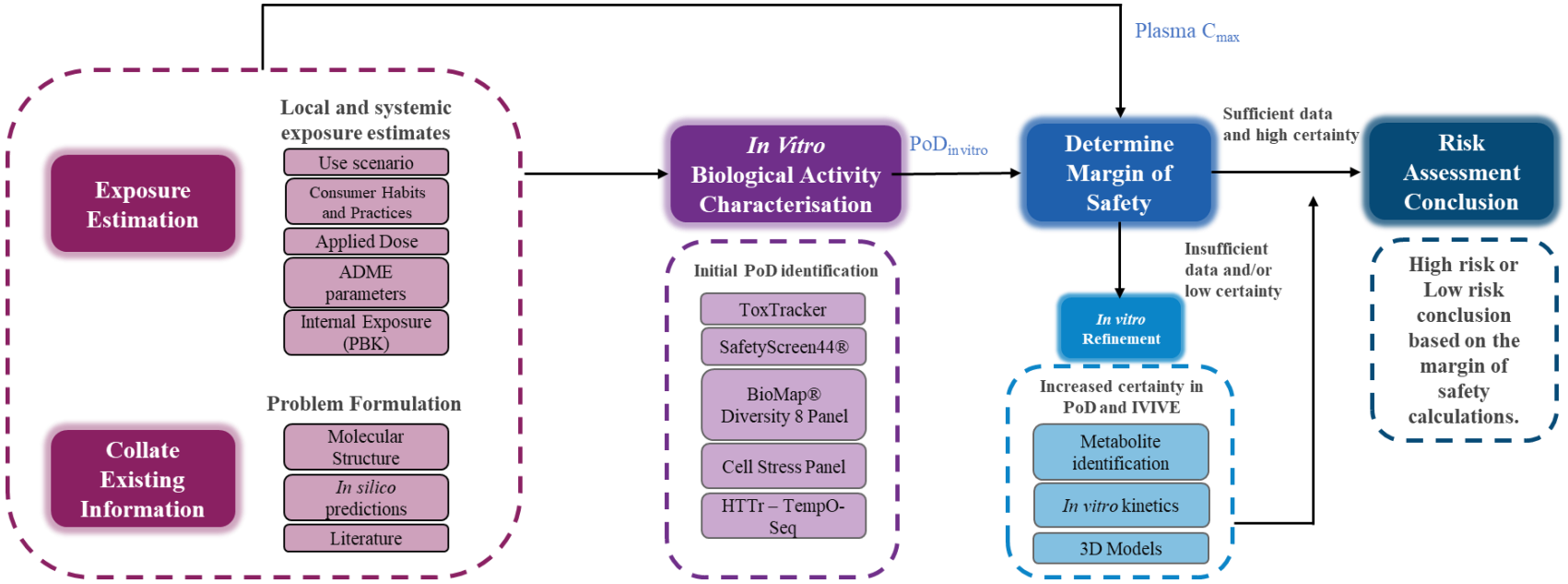
Large BER (i.e., Exposure << POD)

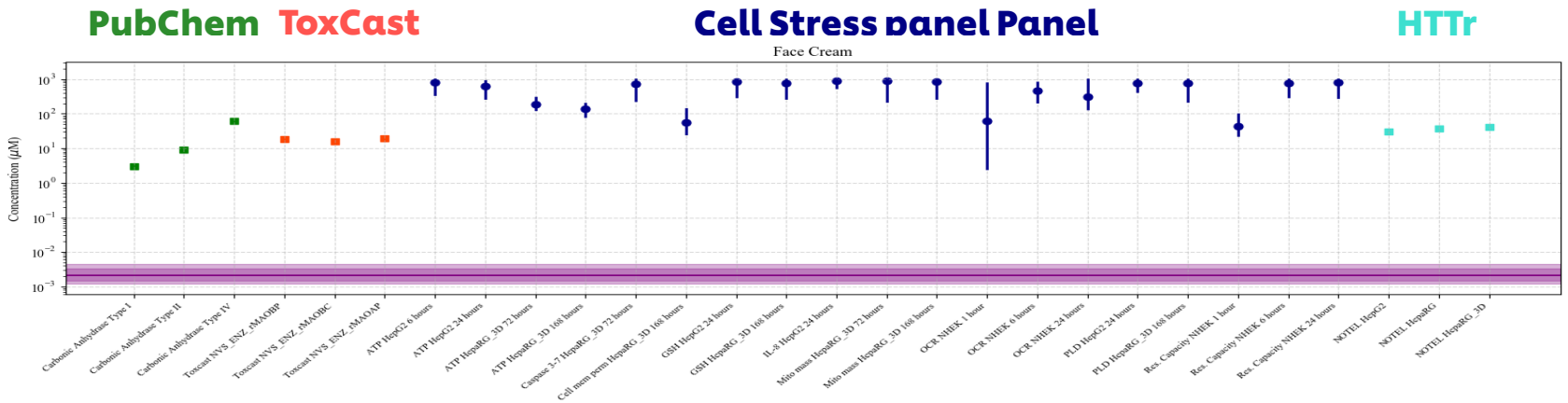
Hypothesis: If a chemical ingredient does not trigger any bioactivity at human relevant exposure levels, then there can be no adverse health effects.

Safety Decision

Gaining confidence in NAMs: first case study with coumarin

For coumarin, a safety assessment based on NAMs was at least as protective as the risk assessment based on traditional approaches

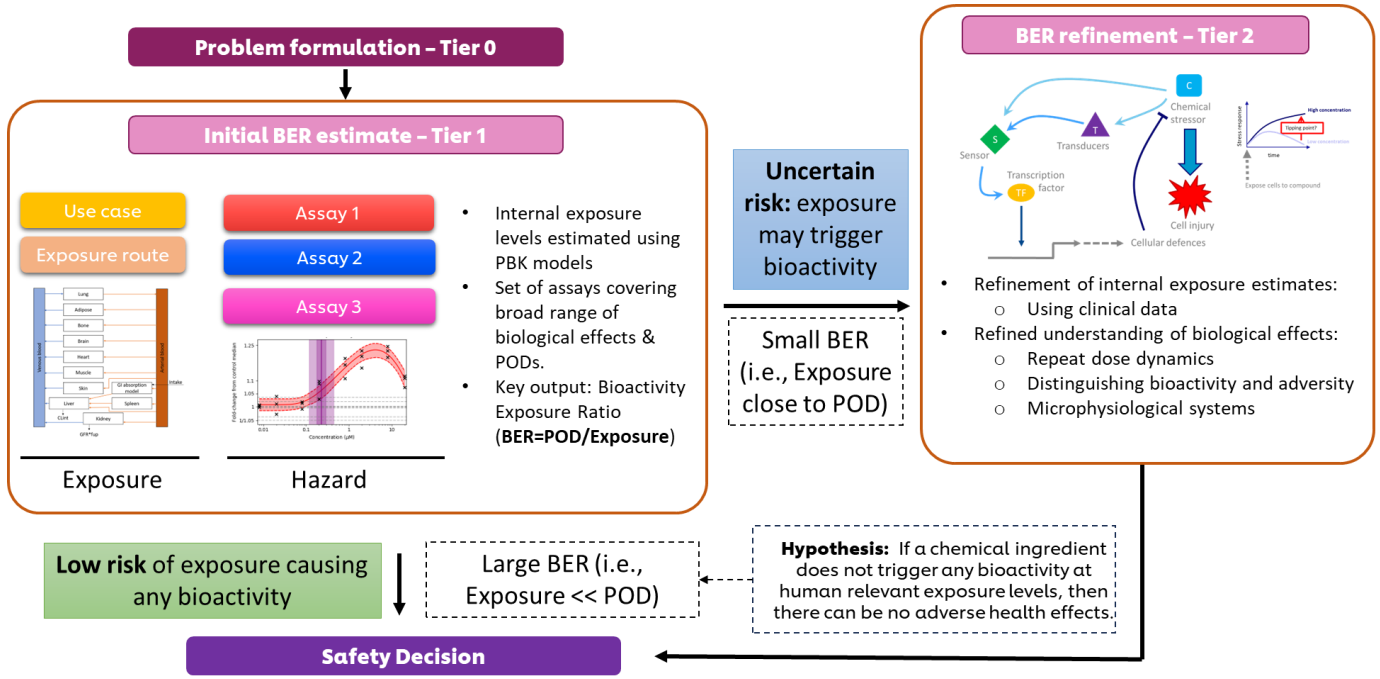




Baltazar *et al.*, (2020) *Tox Sci* Volume 176, Issue 1, 236–252

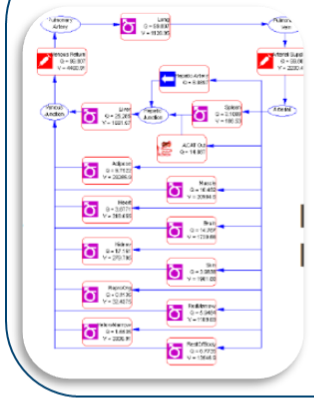
Building and evaluating a systemic safety toolbox

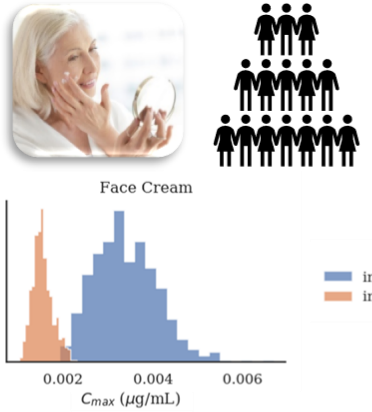
1. Focus the tools and workflows used to make decisions at Tier 1 of an NGRA framework. Includes both:
   - *In vitro* cell assays
   - Exposure models
2. Decisions that can be made with the toolbox are either that a given exposure level is **low risk**, or that the exposure scenario is of **uncertain risk.**
3. In principle, the toolbox will be used as part of a wider tiered assessment framework, which uses e.g., other data through **Tier 0**.



The key NAMs in our NGRA approach

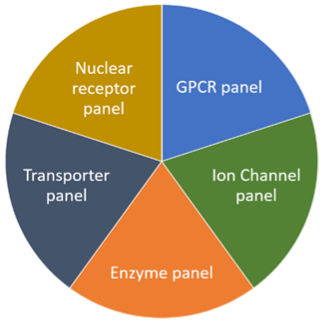
PBK Modelling





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

In vitro pharmacological profiling



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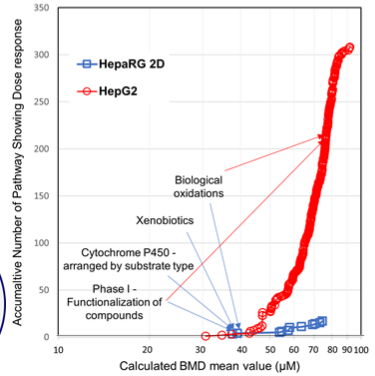
Transcriptomics

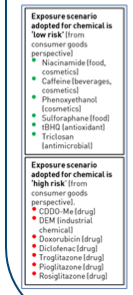
- Use of full human gene panel ~ 21k
- 24 hrs exposure
- 7 concentrations
- 3 cell lines HepG2/ HepaRG/ MCF7
- 3D HepaRG spheroid

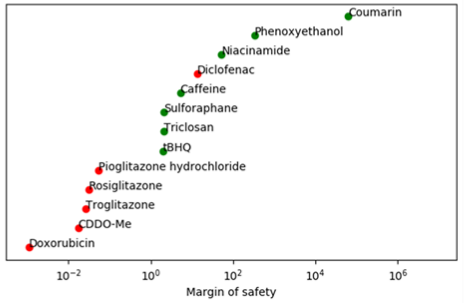
BIFROST
BMDexpress 2



Cellular Stress Pathways

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





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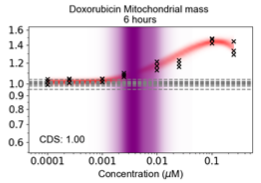

Toxicol Sci (2020), **176**, 11-33

Key aims: 1) select *in vitro* assays that can cover both specific and non-specific mechanisms of toxicity, and 2) can be used to detect early perturbations that may lead to relevant toxicity effects, before the onset of adversity.

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

1. Determine whether the toolbox is fit for purpose
   - Can the toolbox be used to make safety decisions that are protective of human health?
   - Do the various assays and cell types used in the toolbox provide sufficient biological coverage?
   - Are the PBK exposure estimates sufficiently accurate?
2. When evaluating the toolbox, take into account all relevant safety data in assessing the approach:
   - Where available/possible, take into account human safety data.
   - Consider both chronic and acute exposure scenarios
   - Ensure we are protective for a broad range of systemic toxicities.
3. Identify an appropriate safety decision model
   - For example, setting a threshold value on the bioactivity exposure ratio.

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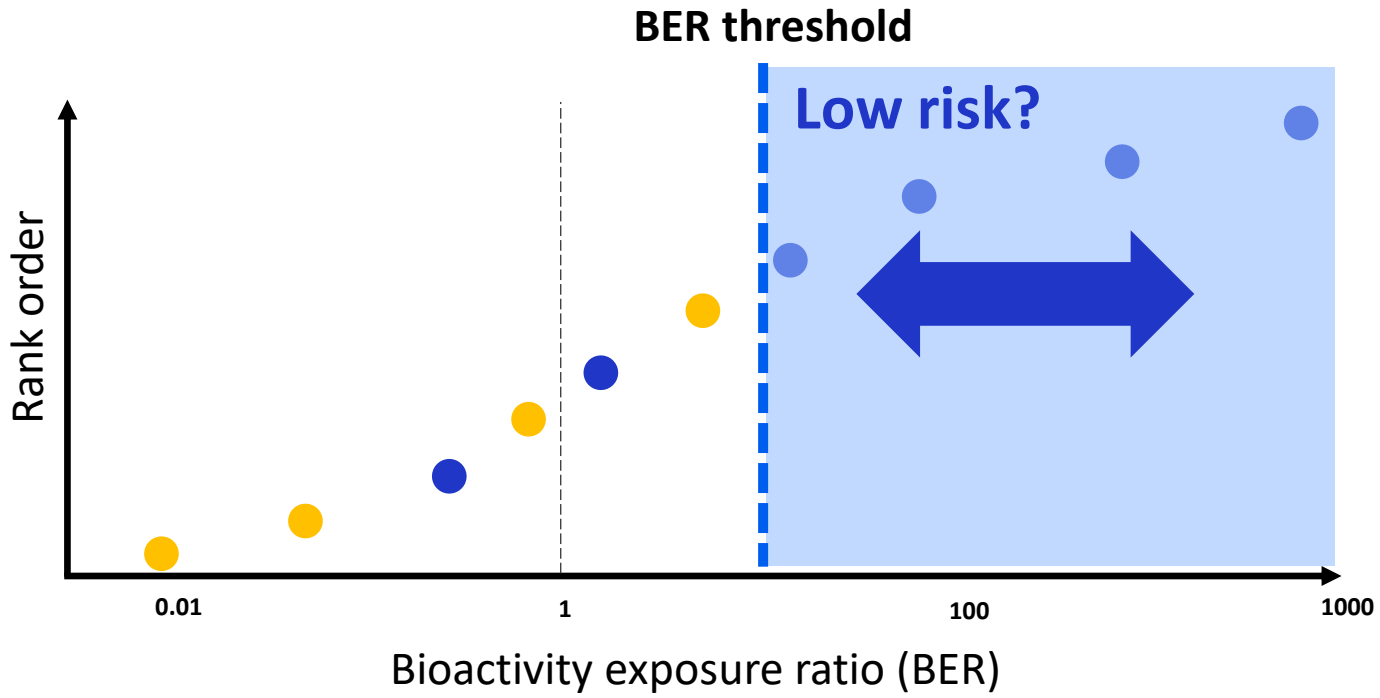
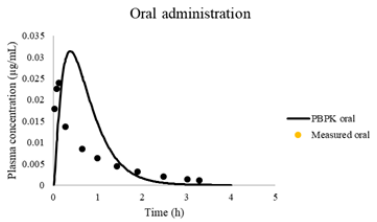
How do we build scientific confidence in a systemic safety toolbox?

Chemical exposures scenarios

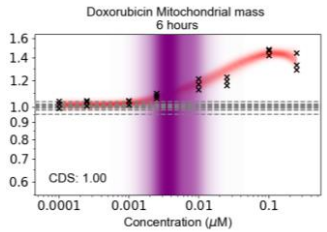
- ‘Low’ risk (from consumer goods perspective) – e.g. foods, cosmetics
- ‘High’ risk (from consumer goods perspective) – e.g. drugs



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

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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 its '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 (extended 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.

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

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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

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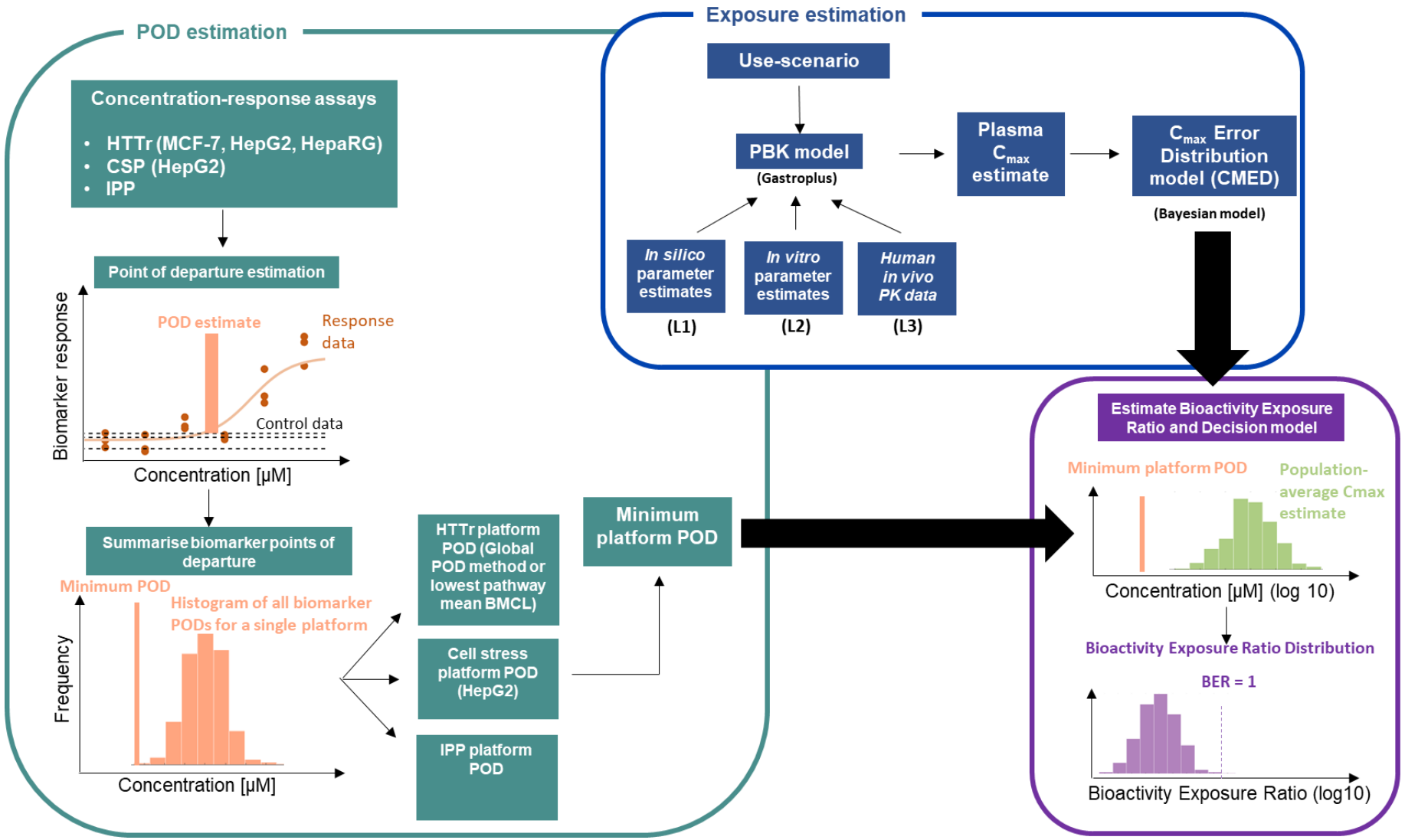
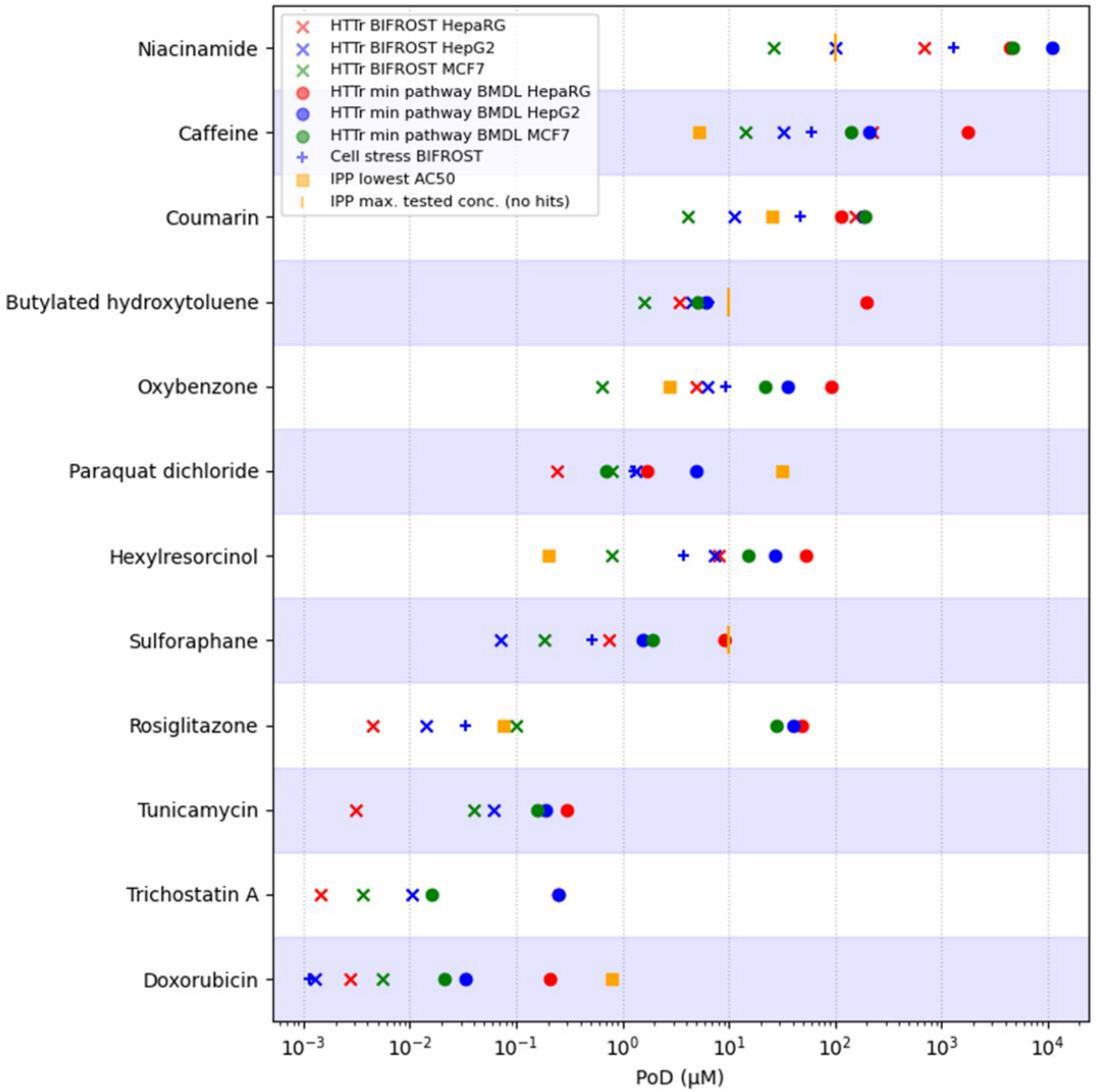
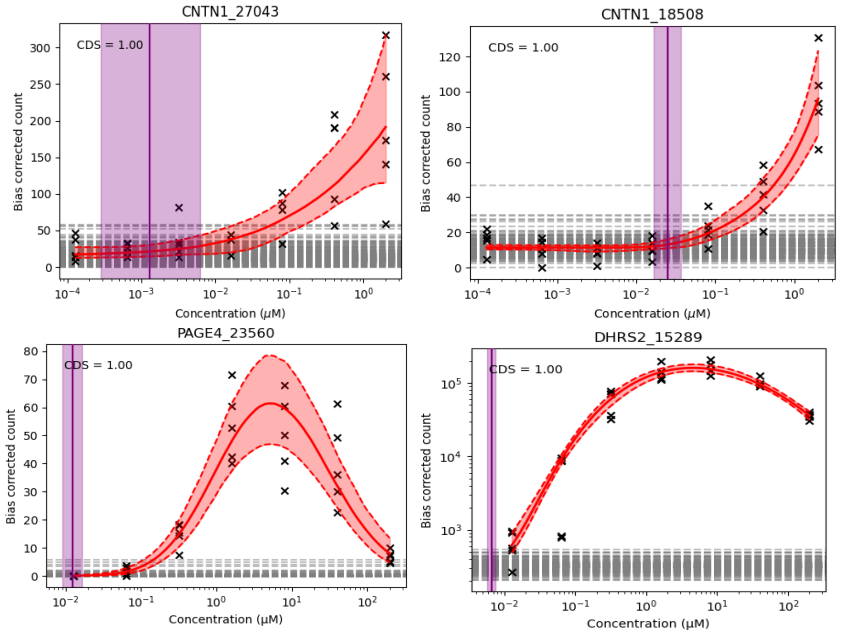
The systemic toolbox workflow to estimating a BER



POD estimation



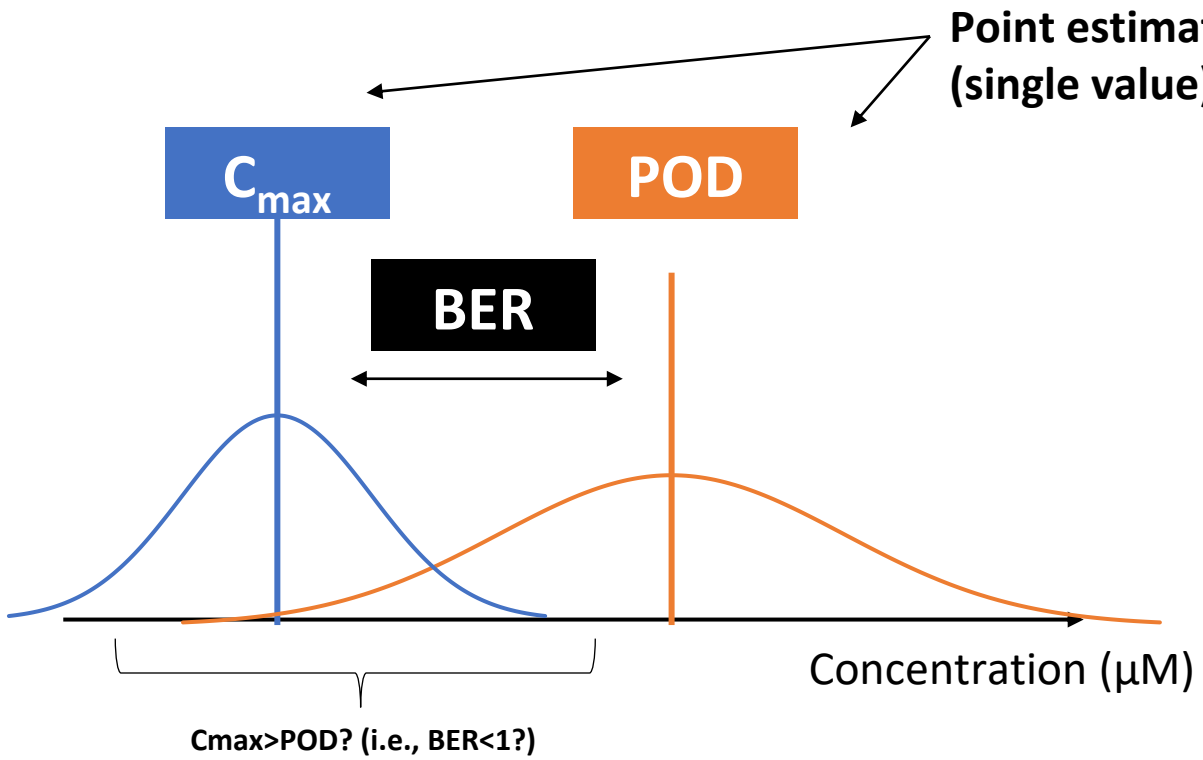


- For 8/10 of compounds tested in the pilot study, HTTr provided the most conservative (lowest POD) when basing the POD on individual genes.
- Pathway based PODs from the HTTr data were typically much higher.

Uncertainty quantification and decision making

Why do we care about quantifying uncertainty?

- Using the point estimates, Cmax appears to be below the POD.
- The true values of both metrics are subject to uncertainty.
- These uncertainties can be captured in terms of distributions.
- The distributions show the range of plausible values for the Cmax and POD.
- Quantifying uncertainty in quantities like Cmax and the POD can be helpful to determine when a safety decision can be made with confidence, or when more refinement is needed.



$$\text{Prob}(\text{BER} > 1)=?$$

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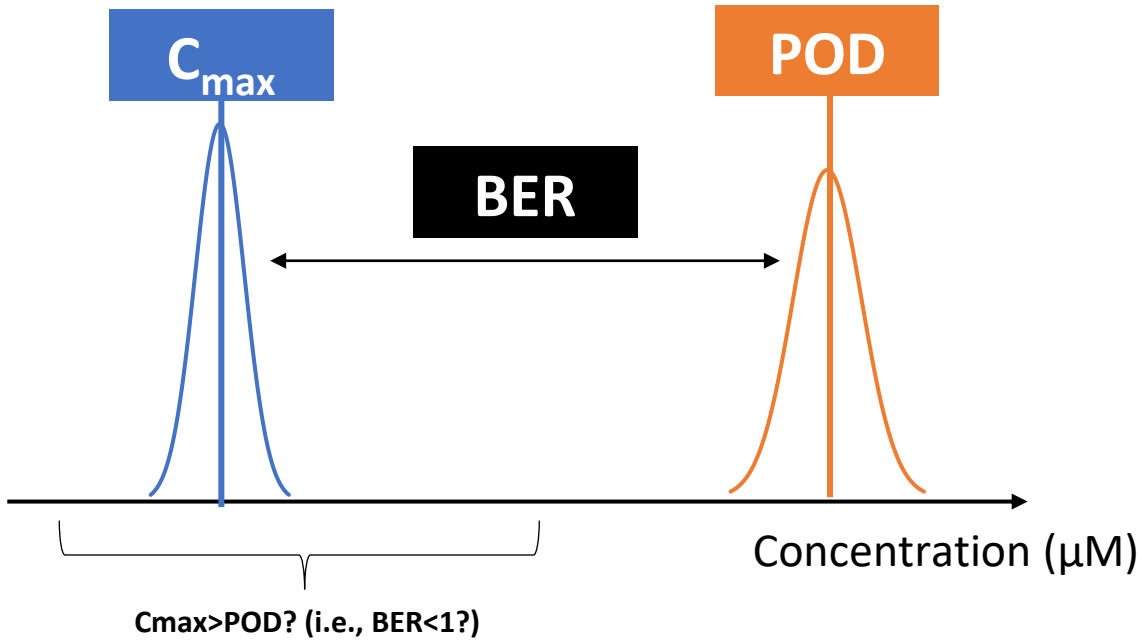
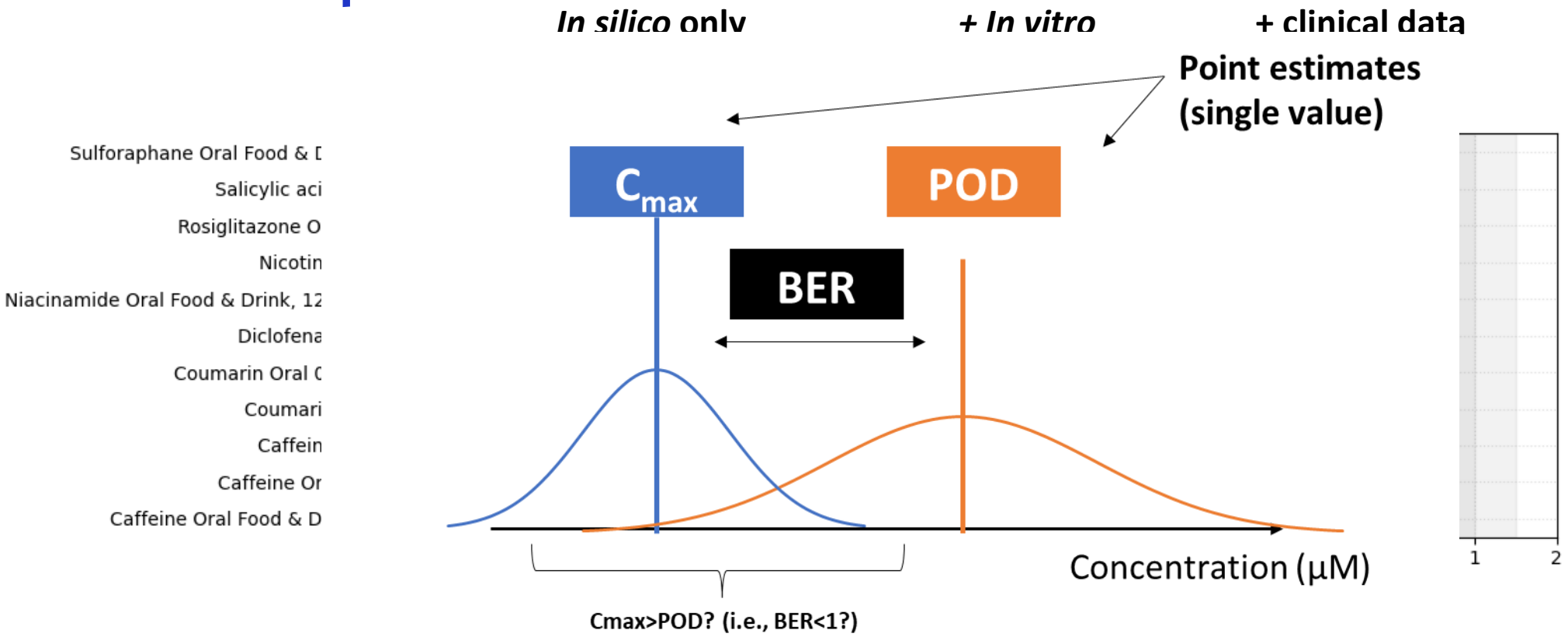


$$\text{Prob}(\text{BER} > 1)=?$$

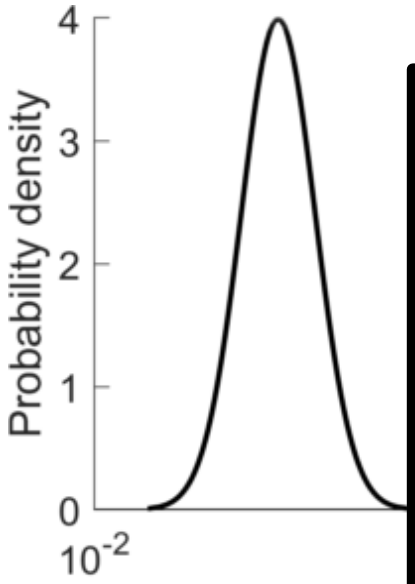
Quantifying PBK model accuracy and uncertainty for different chemical exposure scenarios



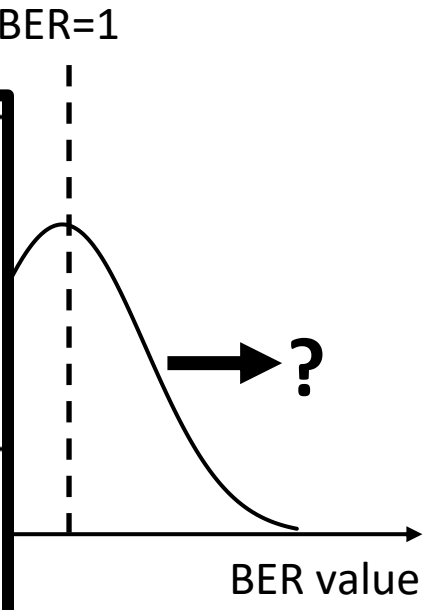
- The accuracy of PBK model Cmax estimates can be quantified by comparing the predicted Cmax value to measured values for different clinical datasets.
- The Cmax Error Distribution (CMED) model was developed using these data to quantify the uncertainty in a PBK Cmax prediction novel substance or exposure scenario, depending on how the PBK model had been parameterised.

Estimating the Bioactivity Exposure Ratio distribution

$\text{Prob}(\text{BER} > 1) \sim 0$

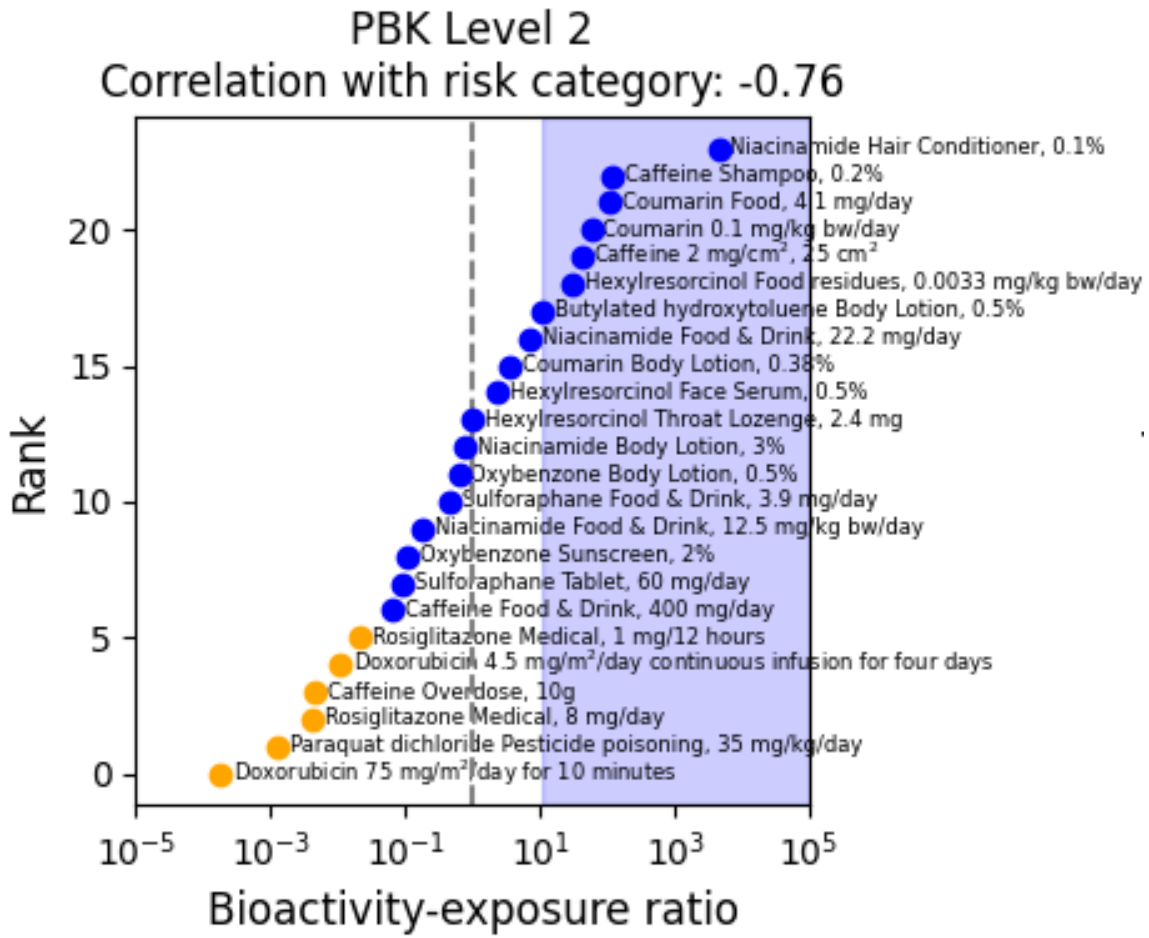


PBK Level	Threshold BER Required for Exposure to Be Identified as Low Risk	Confidence Threshold ($p_{threshold}$) Required for Exposure Scenario to Be Identified as Low Risk
1	110	.98
2	11	.97
3	2.5	.95



- The distribution representing uncertainty C_{max} estimate can be combined with the minimum PODs to form a single BER distribution. (Currently this distribution does not take into account POD uncertainty).
- The minimum POD was selected in order to ensure safety decisions are sufficiently conservative.

Systemic safety toolbox pilot study results: 100% protective for all PBK levels



Blue: low risk chemical-exposure scenario

Yellow: high risk chemical-exposure scenario

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

Across the various PBK parameterisation levels:

- **100% protective** (i.e., 100% of all high-risk exposure scenarios were correctly identified as not low risk)
- **Up to 69% utility** (i.e., 69% of all low-risk exposures were correctly identified as low risk).

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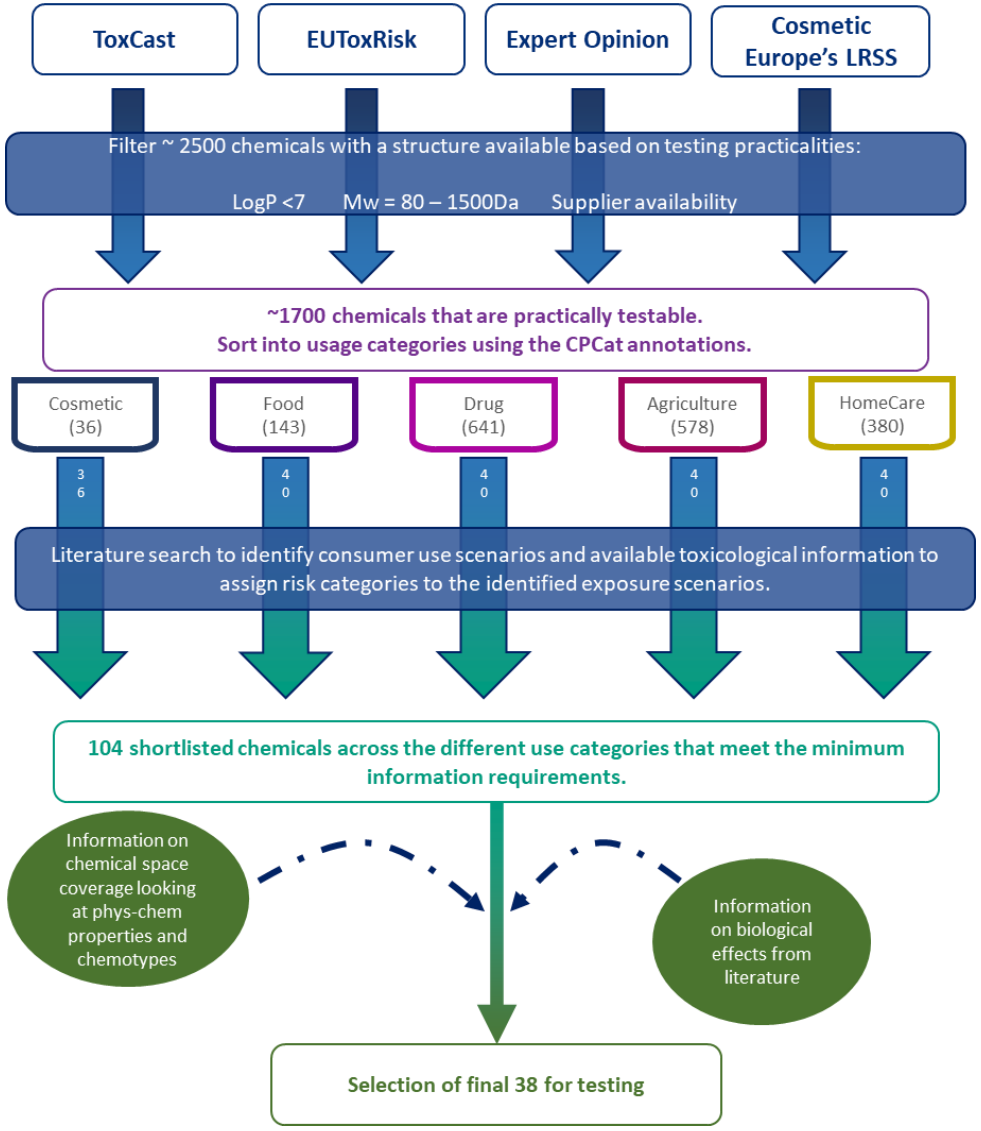
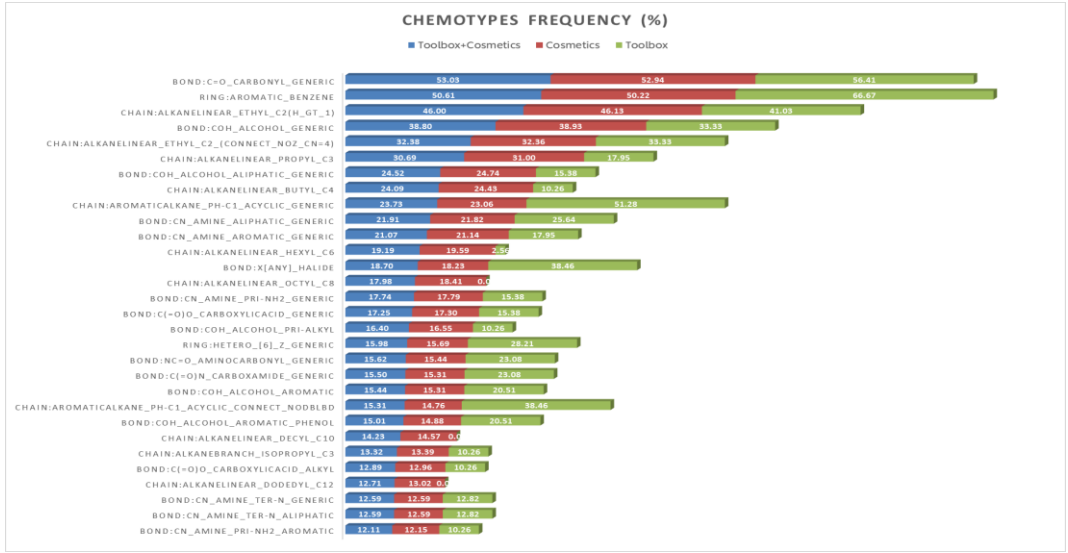
*Middleton et al (2022), *Tox Sci,* Volume 189, Issue 1, Pages 124-147

Expanding the set of benchmark chemical exposure scenarios





- Manual chemical or exposure scenario selection may result in strong biases.
- Therefore, for the extended evaluation, benchmarks were selected using a semi-randomised processed.
- The final set of benchmarks represented a wide range of different potencies, chemotypes and potential toxicity mechanisms.

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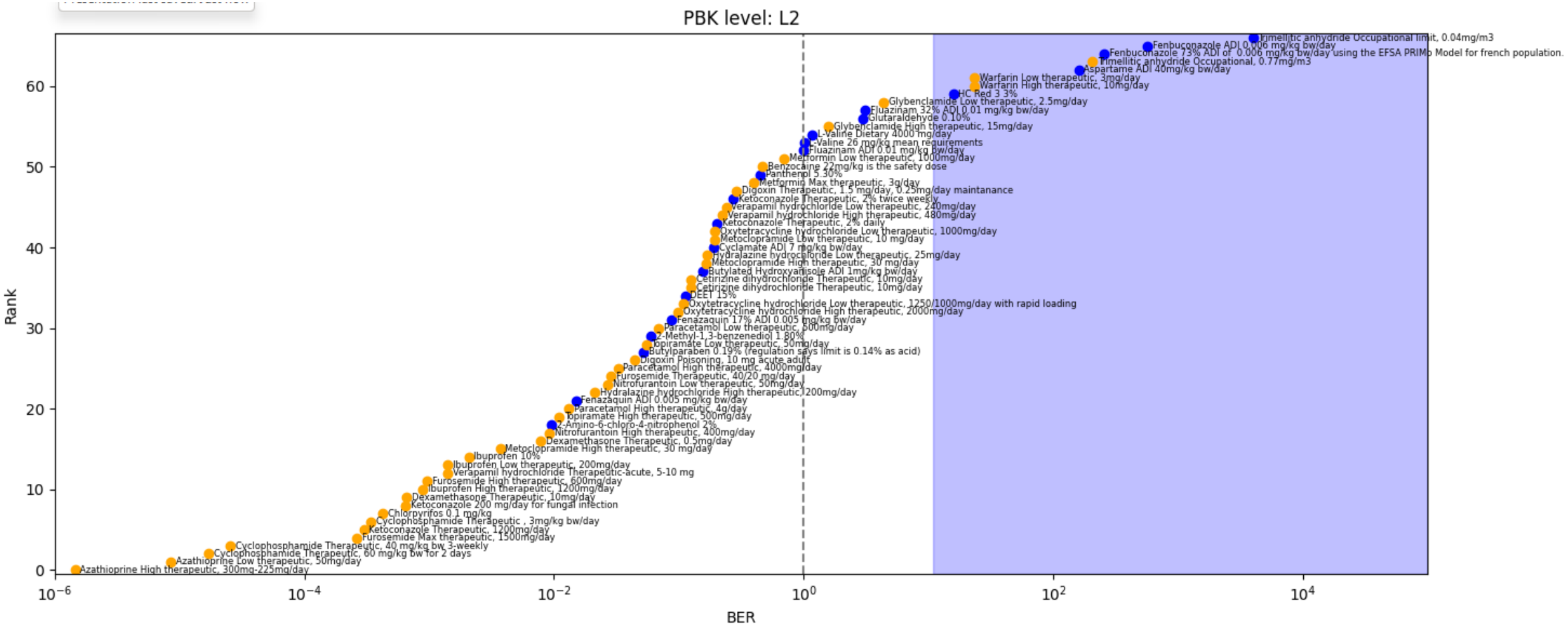
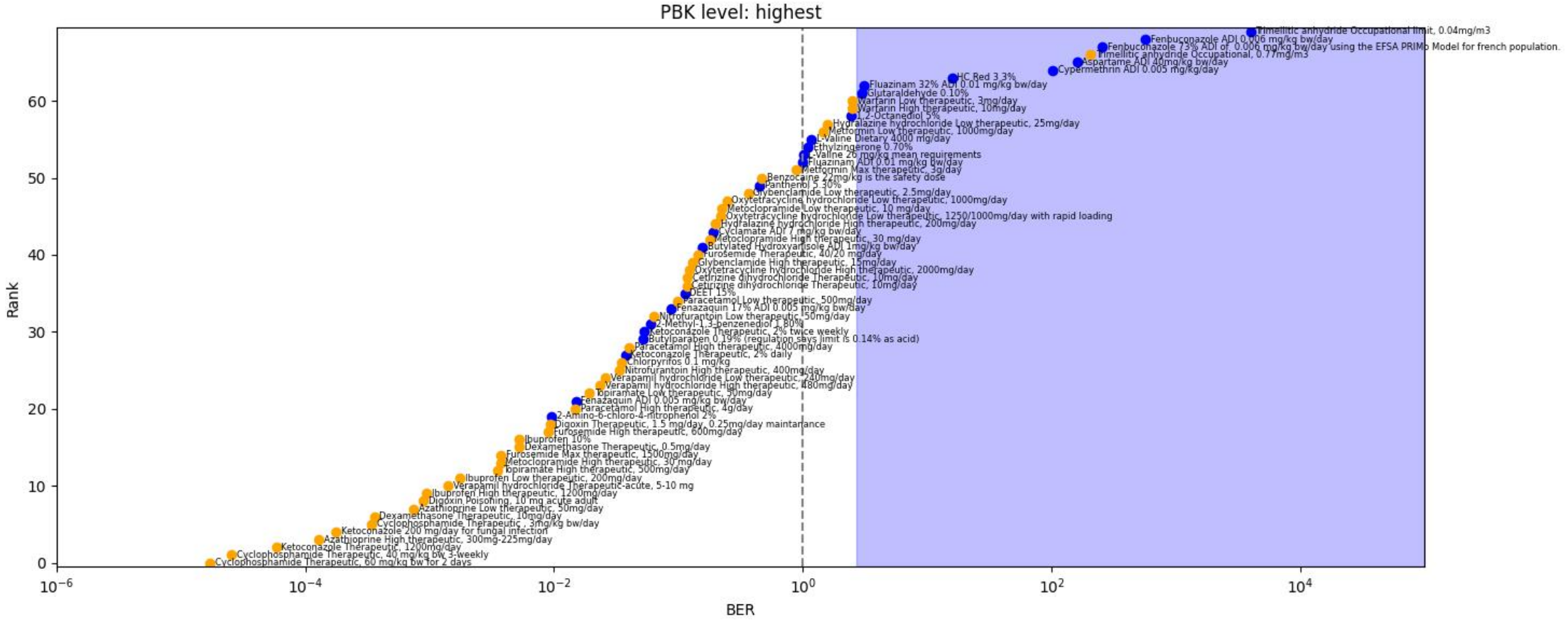
Toolbox performance: PBK L2 exposure estimates



Protectiveness: 93% (43 out of 46)
Utility: 24% (5 out of 21)
Balanced accuracy: 59%

Toolbox performance: Highest available PBK level

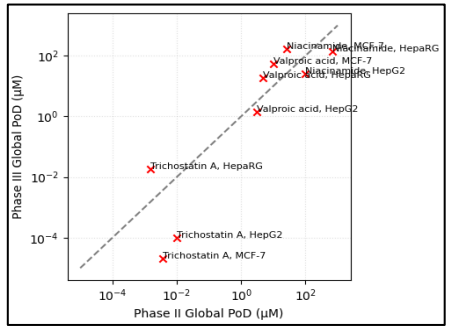


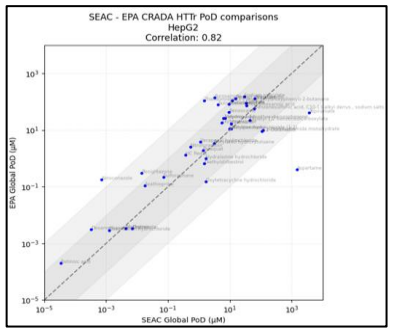
Protectiveness: 98% (45 out of 46)
Utility: 33% (8 out of 24)
Balanced accuracy: 66%

Discussion and next steps

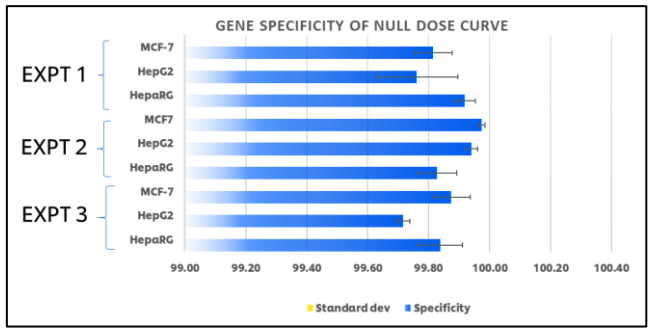
- We have now extended the evaluation to 38 chemicals and 70 exposure scenarios. Protective for 93-98% of scenarios (depending on PBK level).
- **Unilever-EPA CRADA**: Generating data for 10 cell lines, using high-throughput transcriptomics and phenotypic profiling.
- We are continuing to further establishing scientific confidence through a range of activities.

POD reproducibility

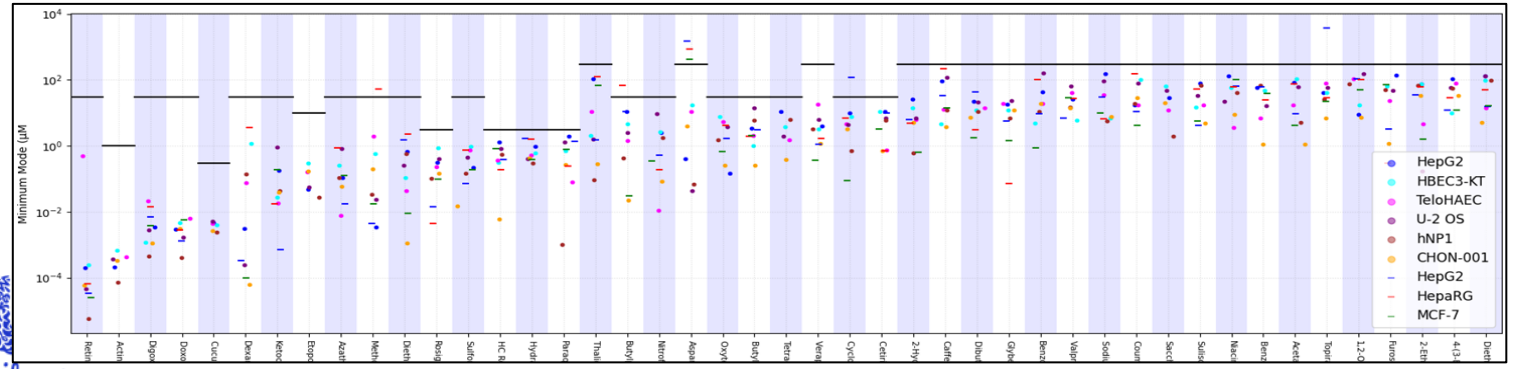


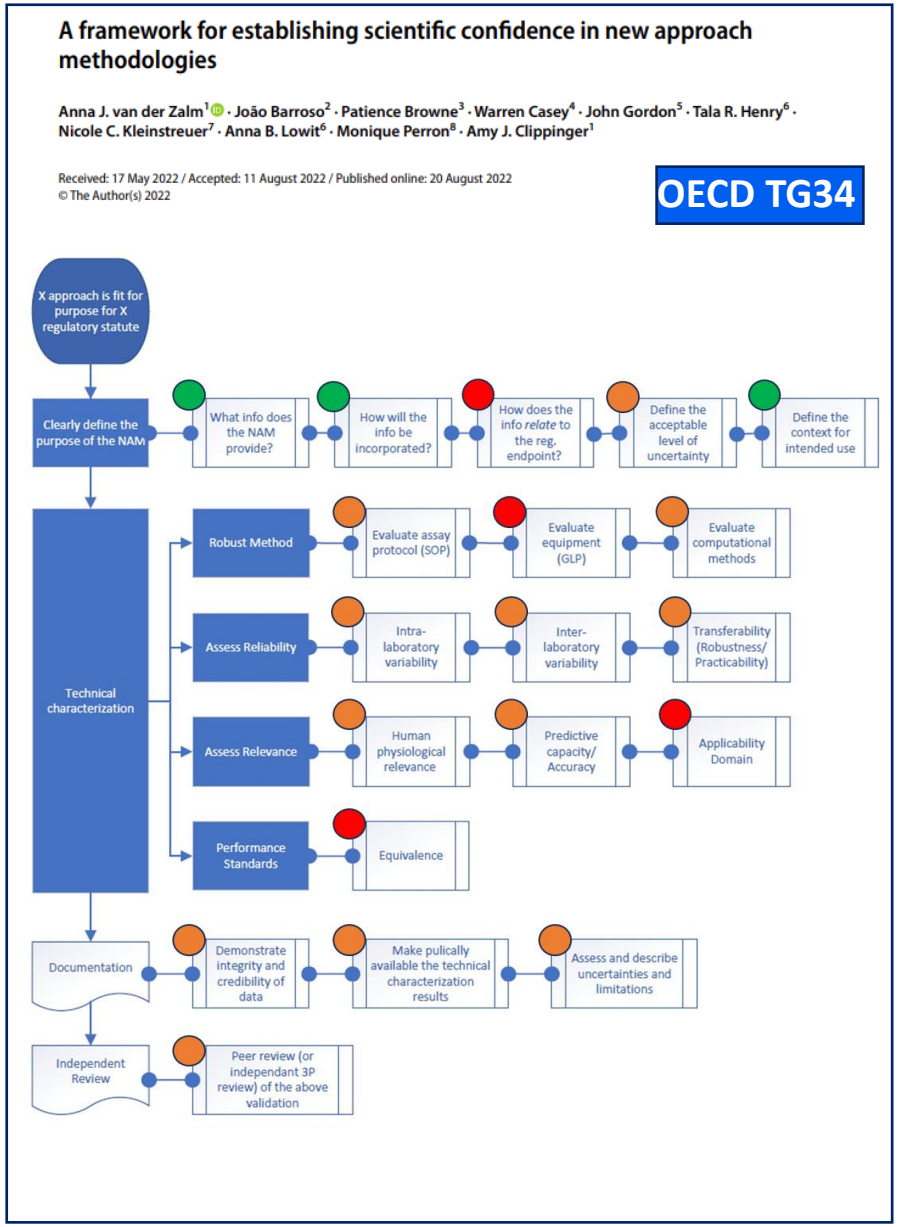


Evaluating different POD methods (sensitivity, specificity etc)



Cell line selection and POD diversity





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Thank You

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