Bioactivity: exposure ratios derived from a systemic NAM-toolbox distinguish between low- and high-risk chemical exposure scenarios
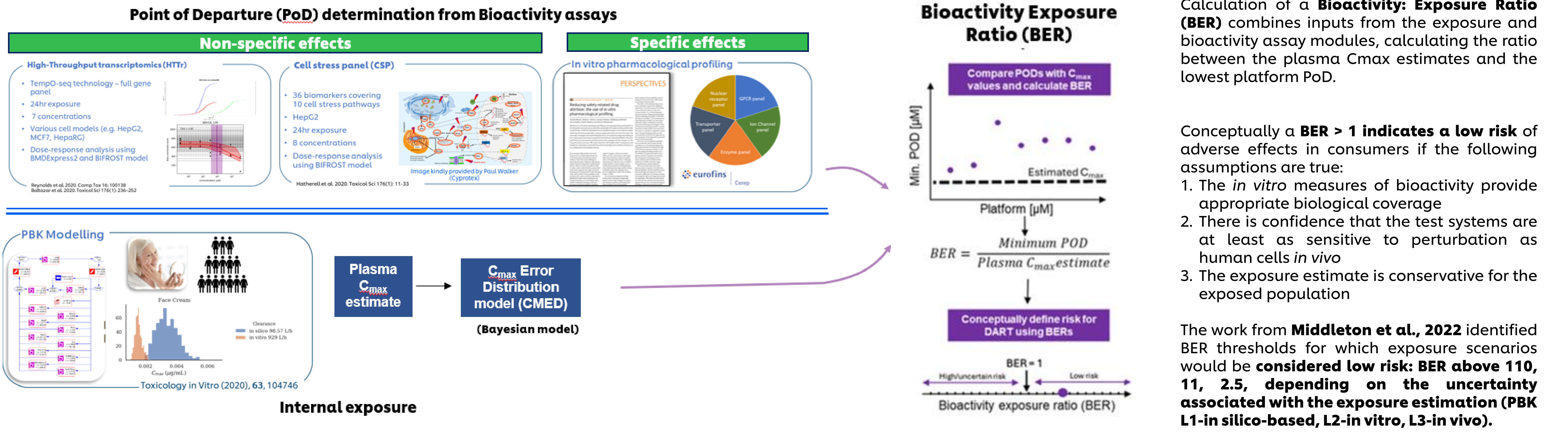
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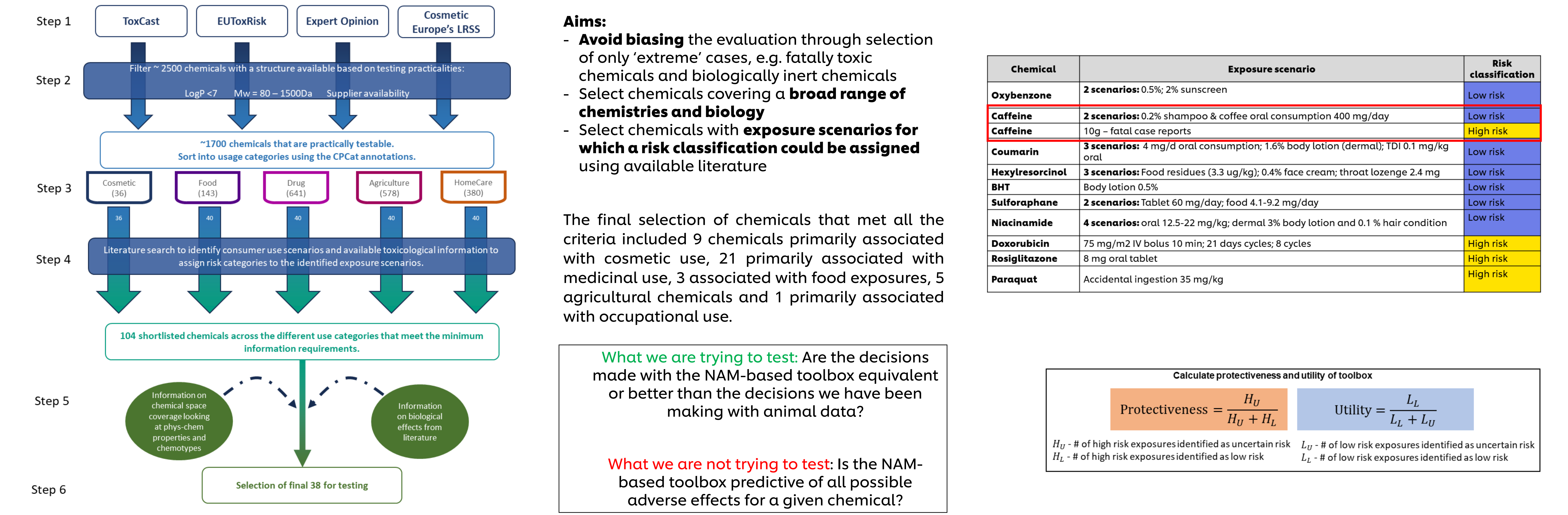
Background

A critical question for risk assessors and regulators is whether safety assessments based on non-animal data can be protective of human health. One important way of establishing scientific confidence in decision making using non-animal methods is through large scale data-driven projects across a broad range of chemistries and biology. Here we show the results of an evaluation activity of a core toolbox of *in vitro* assays and a risk assessment workflow for decision making using benchmark chemical exposure scenarios to interpret the performance of the toolbox and the workflow.

Define Toolbox Components and Perform Proof of Principle Study

Calculation of a **Bioactivity: Exposure Ratio (BER)** combines inputs from the exposure and bioactivity assay modules, calculating the ratio between the plasma Cmax estimates and the lowest platform PoD.

Conceptually a **BER > 1 indicates a low risk** of adverse effects in consumers if the following assumptions are true:
1. The *in vitro* measures of bioactivity provide appropriate biological coverage
2. There is confidence that the test systems are at least as sensitive to perturbation as human cells *in vivo*
3. The exposure estimate is conservative for the exposed population

The work from **Middleton et al., 2022** identified BER thresholds for which exposure scenarios would be **considered low risk: BER above 110, 11, 2.5, depending on the uncertainty associated with the exposure estimation (PBK L1-in silico-based, L2-in vitro, L3-in vivo).**

$$BER = \frac{Minimum\ POD}{Plasma\ C_{max}\ estimate}$$

Select Test Chemicals and Set Performance Criteria

Aims:
- **Avoid biasing** the evaluation through selection of only 'extreme' cases, e.g. fatally toxic chemicals and biologically inert chemicals
- Select chemicals covering a **broad range of chemistries and biology**
- Select chemicals with **exposure scenarios for which a risk classification could be assigned** using available literature

The final selection of chemicals that met all the criteria included 9 chemicals primarily associated with cosmetic use, 21 primarily associated with medicinal use, 3 associated with food exposures, 5 agricultural chemicals and 1 primarily associated with occupational use.

What we are trying to test: Are the decisions made with the NAM-based toolbox equivalent or better than the decisions we have been making with animal data?

What we are not trying to test: Is the NAM-based toolbox predictive of all possible adverse effects for a given chemical?

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

Calculate protectiveness and utility of toolbox

$$Protectiveness = \frac{H_U}{H_U + H_L} \qquad Utility = \frac{L_L}{L_L + L_U}$$

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

Evaluation Results

PBK LEVEL	PROTECTIVENESS	UTILITY
L1	93%	8%
L2	93%	27%
L3	98%	0%
Highest	96%	29%

Traditional PoDs vs. NAM PoDs (mg/kg bw/day)
PBK level: highest
Pearson correlation: 0.57

References:

Cable et al., 2025 Tox Sci 204(1):79-95.
Middleton et al., 2022 Tox Sci 189(1):124-147.

- The NAM-based toolbox can be used to make decisions that are protective of human health in at least 93% of cases, despite not predicting the MoA.
- The current proposed toolbox is intended to sit within a tiered risk assessment framework and does not differentiate bioactivity from adversity at this stage. The observed low utility could be addressed by the incorporation of further testing or more detailed interpretation of the Tier 0 (in silico tools and available literature) and Tier 1 (NAM-based toolbox) results.
- More chemicals should be tested to build upon the reference database that currently includes 38 chemicals and 70 benchmark exposure scenarios to increase confidence in the applicability of this approach.