Developing Non-Animal Frameworks for Systemic Safety decisions

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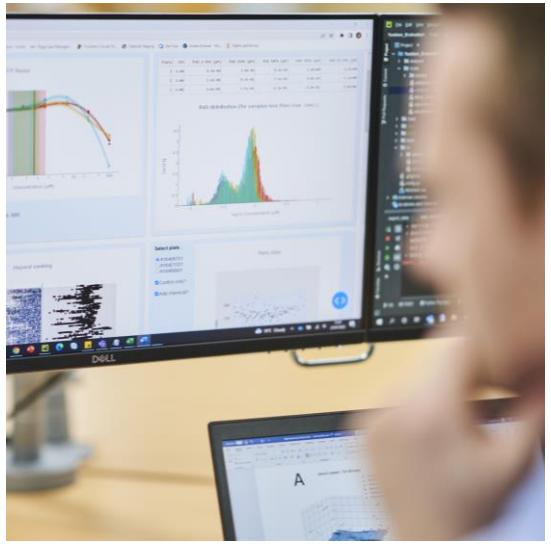

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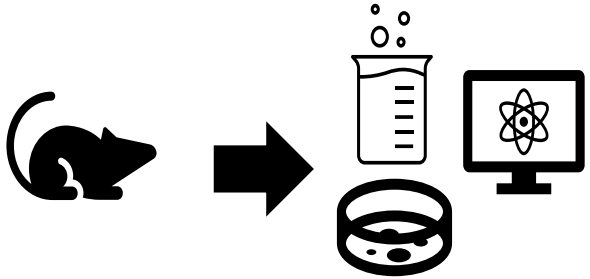
Safety without animal testing - Next Generation Risk Assessment (NGRA)

*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***

Dent et al 2018. Computational Toxicology Volume 7, August 2018, Pages 20-26

* In this presentation the NAMs acronym is used as "non-animal NAMs"

Developing Non-Animal Protective Frameworks for Safety decisions



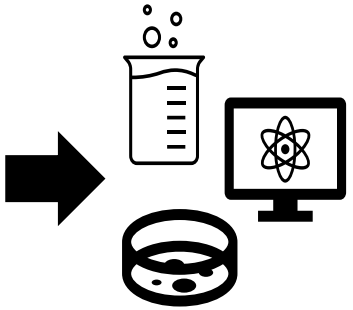
Non-animal NAMs strategies for 1-2-1 replacement – prediction of animal outcome

Prediction of an animal test is not necessarily relevant to assess human safety

The rodent studies have been used in a protective manner with the use of uncertainty factors rather than in a predictive way

AOPs (currently 438 in AOP wiki)

A-O-P-Wiki



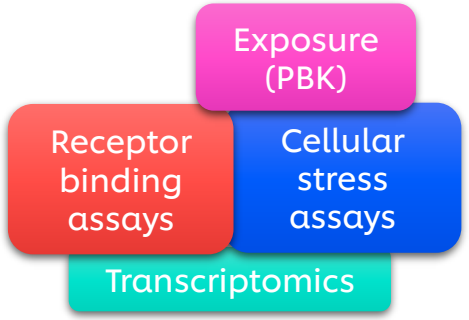
Development of battery of assays aligned to AOPs

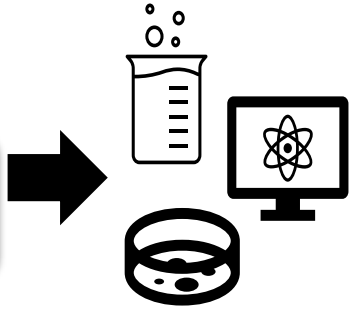
Not feasible as a tier 1 approach

~ 1000 of assays need to be if multiple AOPs are identified

Critical question is: how to identify the relevant AOP?

Useful for Tier 2/bespoke safety assessment when differentiation between bioactivity & adversity is needed





Development of high-throughput & broad coverage set of non-animal NAMs

Hypothesis:

If biological activity measured using a broad suite of human-relevant test systems is above the predicted exposure in humans, then there are no systemic adverse effects.

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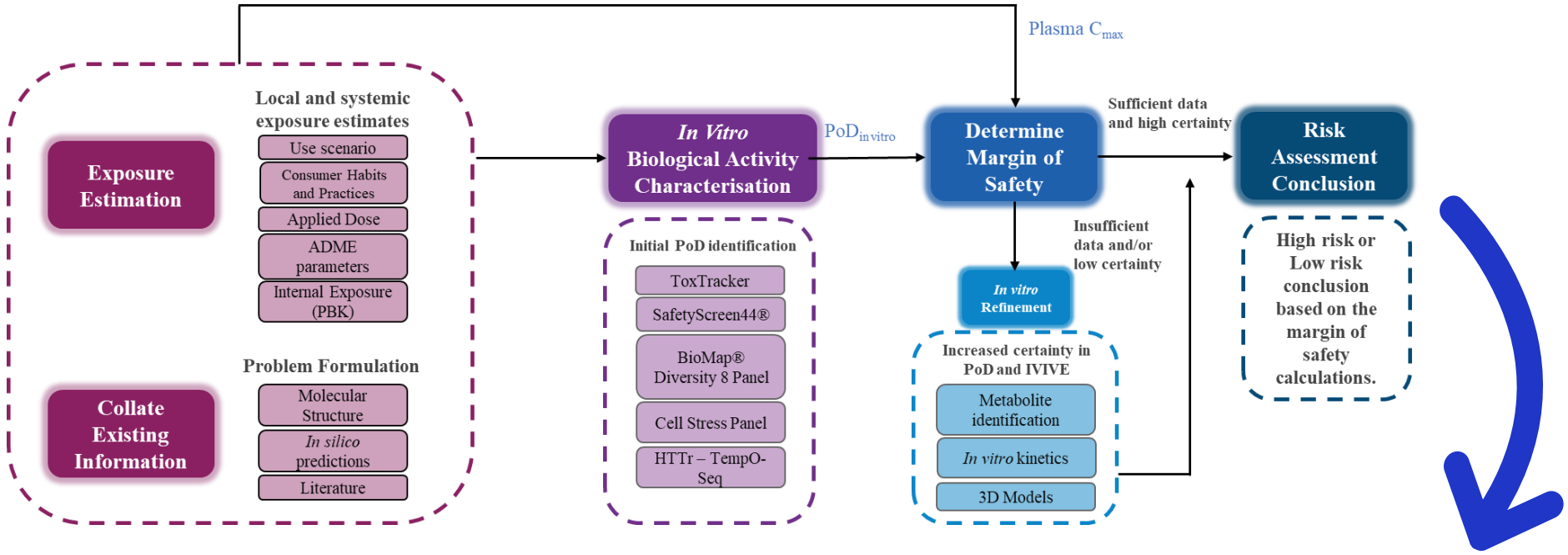
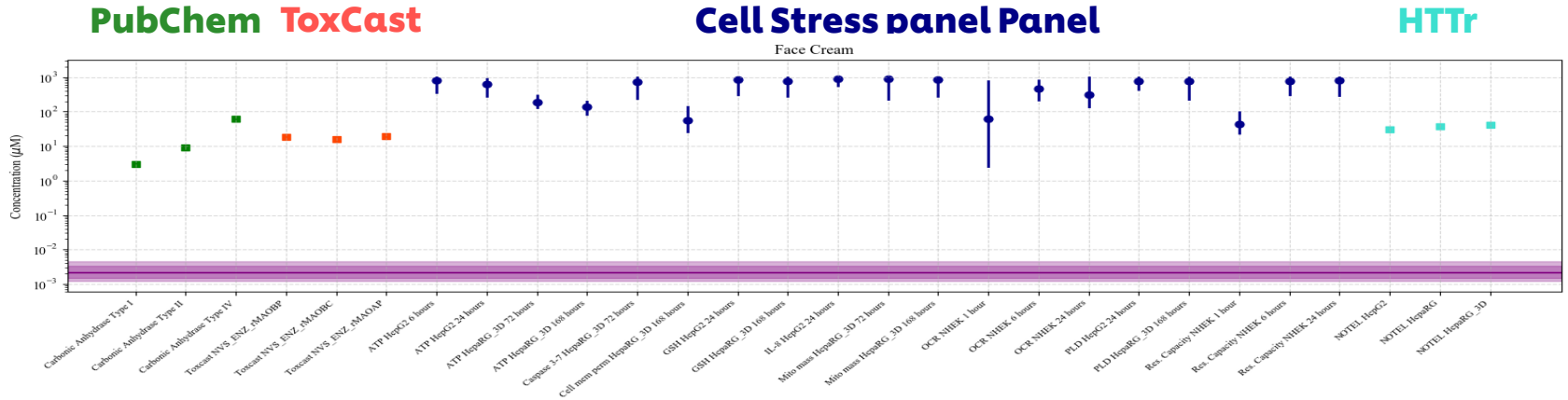
Gaining confidence in NAMs: first case study with coumarin

For coumarin, a safety assessment based on non-animal approaches 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

Gaining confidence in a systemic toxicity NAM toolbox – benchmarking with historical safety decisions for 10 chemicals and 24 exposure scenarios

Alistair Middleton talk
Session S404
(Symposium)
Wednesday, August 30, 14:00 – 16:00

Selection of the non-animal NAMs

- **Human Exposure :**
  - Internal exposure – PBK modelling to derive plasma C_{max}
- **Bioactivity NAMs**
  - **In vitro pharmacological profiling** (63 targets with known safety liabilities) – IC50 derivation
  - **Cell stress panel** in HepG2: The panel comprised biomarkers that cover 8 key stress pathways, mitochondrial toxicity, and cytotoxicity
  - **High-Throughput transcriptomics** (HTTr, TempO-Seq) in MCF7, HepaRG, HepG2 cells.

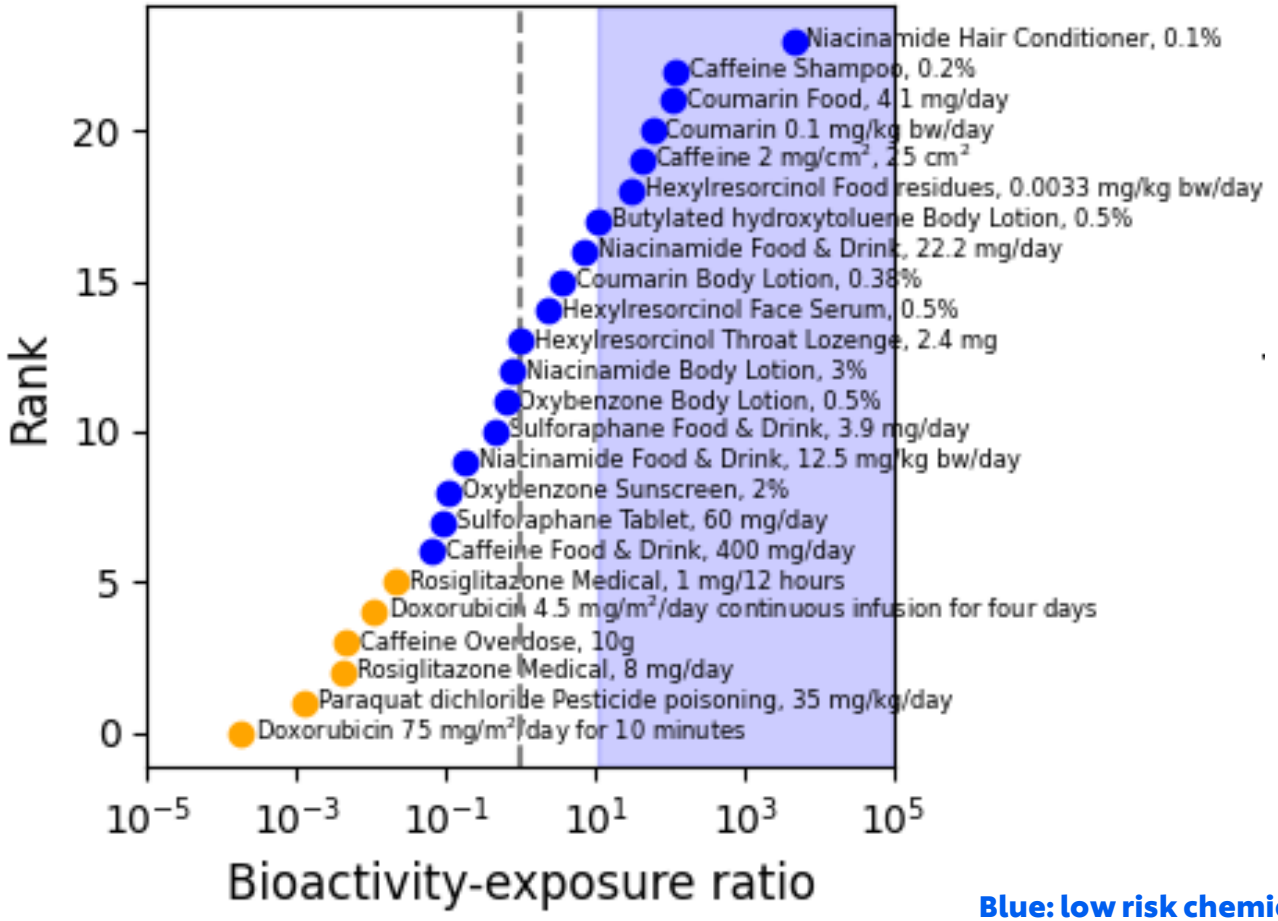
Selection of chemicals and exposure scenario

- Chemicals with well-defined human exposures
- Traditional safety assessment available
- High certainty in the risk classification for each chemical-exposure scenario
- Risk class is relative to consumer health

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
Paraquat	Accidental ingestion 35 mg/kg	High risk

Middleton AM et al (2022). Are Non-animal Systemic Safety Assessments Protective? A Toolbox and Workflow. Toxicological Sciences, 189:124-147.

100% protective for high-risk chemical exposure scenarios



- **Not all low-risk scenarios would be supported with this toolbox**
- **Very conservative safety decisions using Tier 1 toolbox alone**

Blue: low risk chemical-exposure scenario
Yellow: high risk chemical-exposure scenario

Blue shaded region BER> 11

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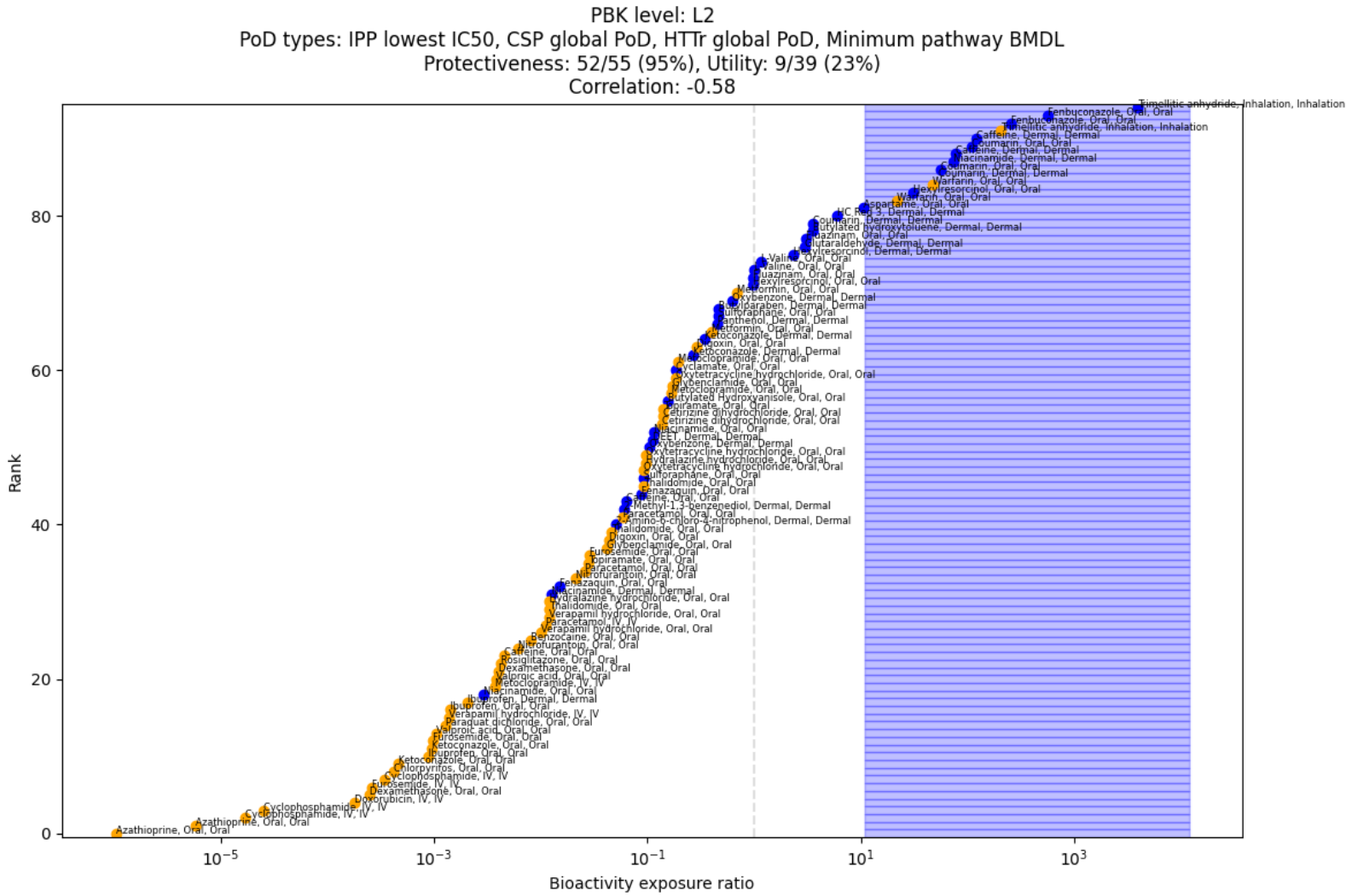
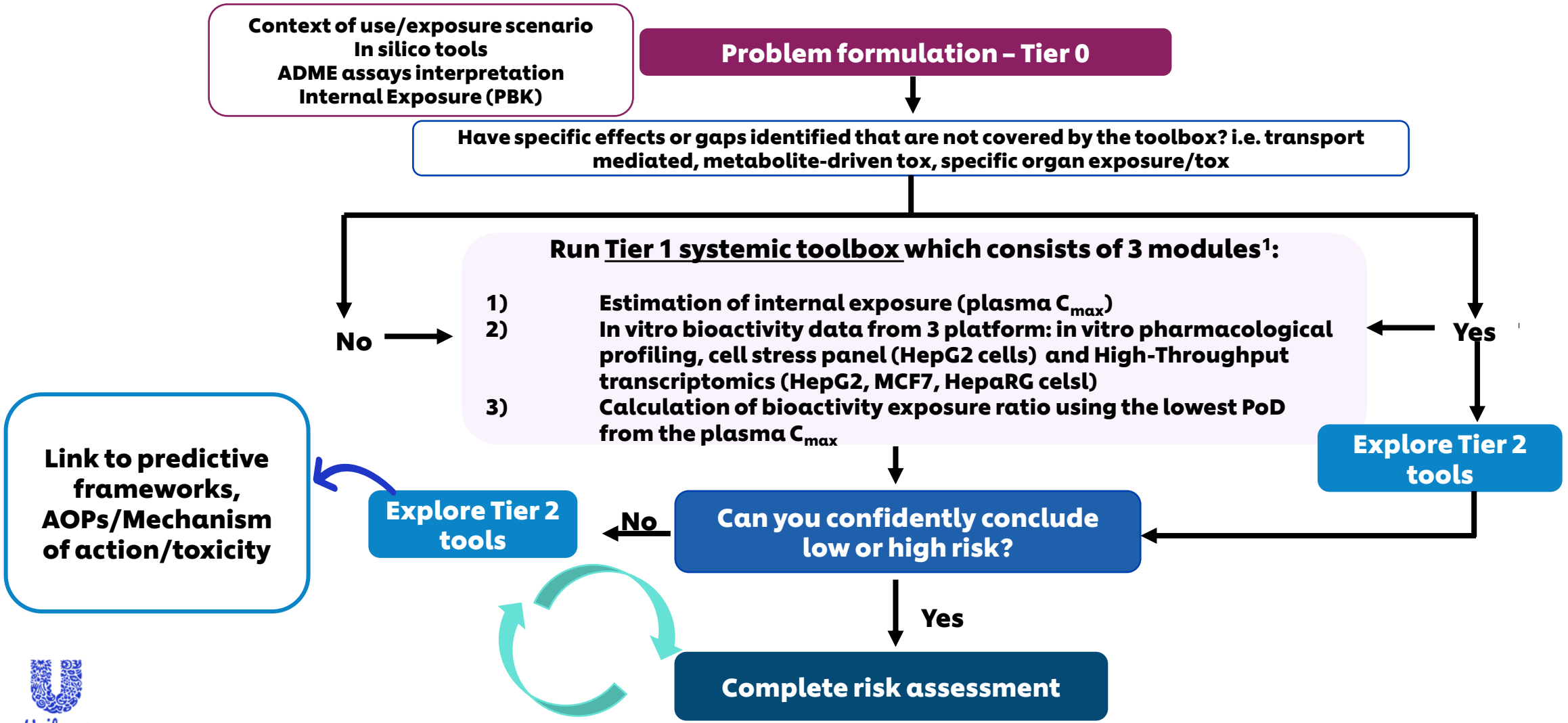
NAM toolbox remains protective (95%) when 38 additional chemicals and 60 exposure scenarios were tested



- **Toolbox not protective for 3/55 of the high-risk exposure scenarios**
- **Exposure scenarios not protective for:**
  - Warfarin therapeutic oral dose
  - Trimellitic anhydride inhalation exposure
- **Using BER >11, only 23% of the low-risk chemical-scenarios would be correctly identified as such**
  - For the other 77%, refinement by using approaches to distinguish bioactivity from adversity would be needed.

*BER > 11 from Middleton et al., 2022

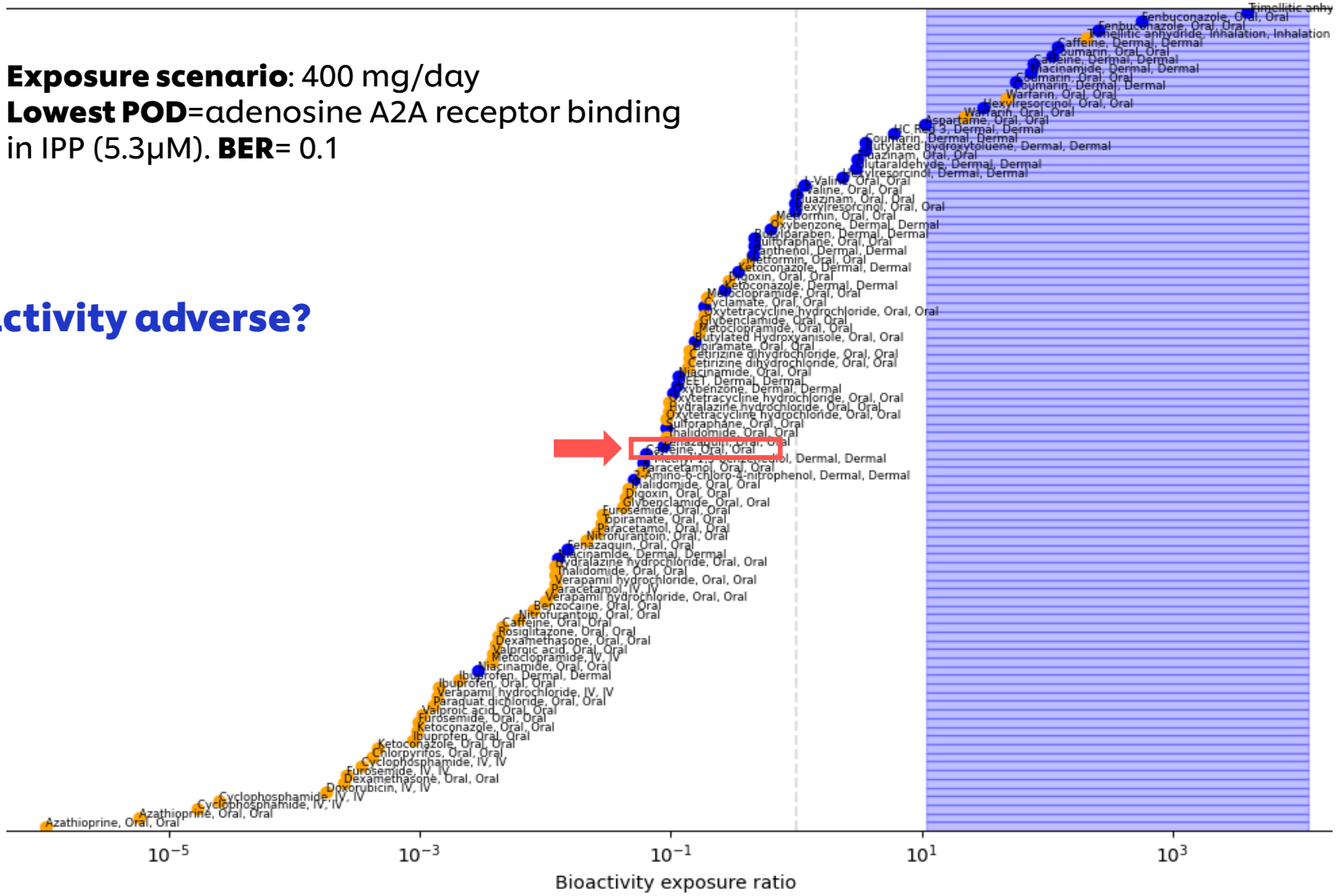
How does the toolbox fit within a Next generation Risk assessment framework?



Example of an ongoing case study: Caffeine in energy drinks

Exposure scenario: 400 mg/day
Lowest POD=adenosine A2A receptor binding in IPP (5.3µM). **BER**= 0.1

Is this bioactivity adverse?



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Example of an ongoing case study: Caffeine in energy drinks

Target Safety review for Adenosine receptor

- Biological interaction and pathways
- Tissue distribution and expression
- Physiological role of the target
- Similarity across the species
- Disease or pathology association.
- Phenotypes of target knockout or transgenic models
- Preclinical or clinical findings with chemicals with the same mode of action
- Chemicals that interact with the target

Target Safety review identified key areas of safety concern

Cardiovascular system

Haematological effects

Neurological effects

Direct: arrythmia, tachycardia

Indirect: via endothelial cells, hypertension

ADORA2A functional antagonism assay
ADORA1 dose response binding assay
ADORA1 functional antagonism assay
Cardiomyocyte FLPR dose response assay (Ca2+ transients in hiPSC-derived cardiomyocytes

Safety assessment approach: Comparison to other methylxanthines in foods and drugs:

- **Theophylline**
- **Pentoxifylline**
- **Theobromine**
- **Others?**

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Conclusions

- **A core toolbox of NAMs** (in vitro and computational) for exposure and bioactivity (potency) can be used to provide BERs which appeared to enable **protective systemic safety decisions to be made without using any animal data.**
- **From the total chemicals tested, 48** so far between test set and evaluation set, the Tier 1 **toolbox was not protective for only 2 chemicals**, warfarin and trimellitic anhydride
- **Decisions made on the tier 1 toolbox alone are very conservative**-> for some chemicals differentiation between bioactivity and adversity is needed for it to be useful
  - ❖ Other authors found similar results i.e., safety decisions from in vitro NAMs more conservative than animal approaches[1,2]
- AOPs and predictive approaches are useful in the context of defining thresholds for adversity
- **The two approaches of protection and prediction coexist in a NGRA framework,** but they need to be fit for purpose

[1]Paul-Friedman et al., 2020. Toxicol Sci. 2020 Jan 1; 173(1): 202–225. [2]Chen Z et al., 2020 ALTEX. 37(4): 623–638.

Acknowledgements

Toolbox team

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Caffeine team

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Thank You

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