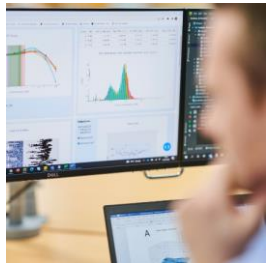
Next Generation risk assessment:

From concept to application

Maria Baltazar, Unilever Safety, Environmental & Regulatory Science , UK

SERS
Safety, Environmental & Regulatory Science



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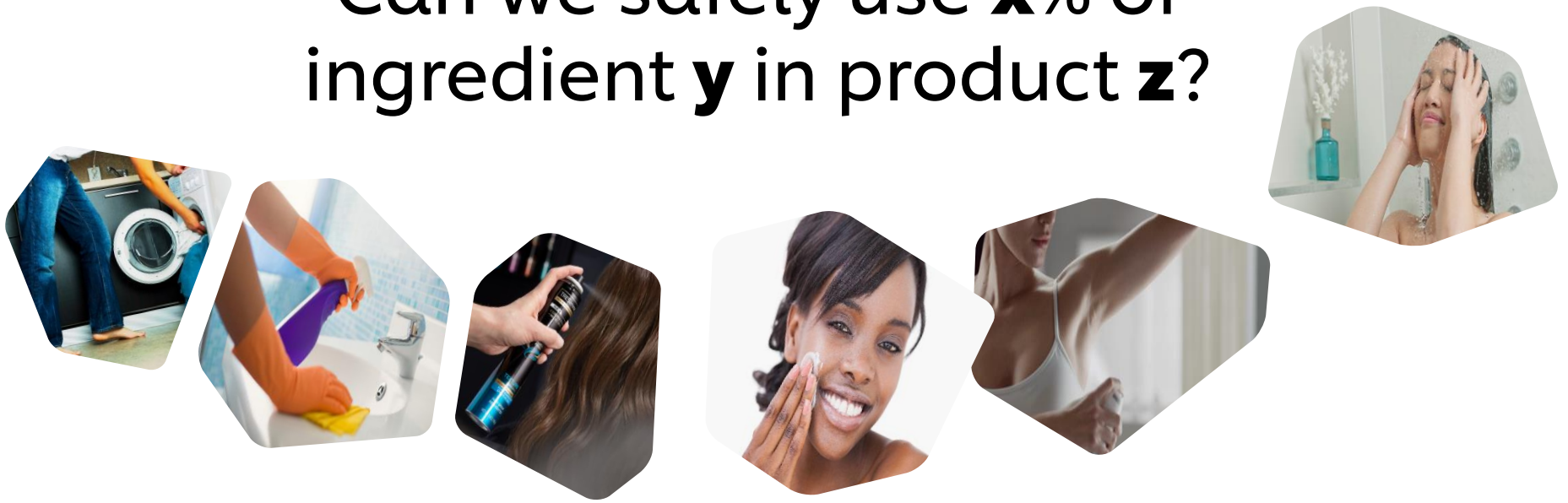
Outline

- Introduction to Next generation risk assessment (NGRA)
- Ongoing efforts to develop systemic toxicity NGRA approaches
- Unilever approach to developing an early tier NAM-systemic toolbox and workflow
- Application of NGRA principles to a case study with climbazole in a face cream product.

The objective of a consumer product risk assessment is…

Can we safely use **x**% of ingredient **y** in product **z**?



All safety assessments of cosmetic ingredients are exposure-driven:

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Introduction to 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[1]

New approach methodologies (NAMs)[2] can be defined as any *in vitro*, *in chemico* or computational (*in silico*) method that when used alone, or in concert with others, enables improved chemical safety assessment through more protective and/or relevant models and as a result, contributes to the replacement of animals.

[1]Dent et al 2018. Computational Toxicology Volume 7, August 2018, Pages 20-26.
[2]Sewell F et al., 2024. 2024 Mar 25;13(2):tfae044. doi: 10.1093/toxres/tfae044

Principles of NGRA from ICCR

4 Main overriding principles:
- The overall goal is a human safety risk assessment
- The assessment is exposure led
- The assessment is hypothesis driven
- The assessment is designed to prevent harm

3 Principles describe how a NGRA should be conducted:
- Following an appropriate appraisal of existing information
- Using a tiered and iterative approach
- Using robust and relevant methods and strategies

2 Principles for documenting NGRA:
- Sources of uncertainty should be characterized and documented
- The logic of the approach should be transparent and documented

Dent *et al .*, (2018) *Comp Tox* 7:20-26

NGRA: The overall goal is a human safety risk assessment

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

"Advances in toxicogenomics, bioinformatics, systems biology, and computational toxicology could transform toxicity **testing from a system based on whole-animal** testing **to one founded primarily on *in vitro* methods** that evaluate changes **in biologic processes using cells, cell lines, or cellular components, preferably of human origin**." 2007

Tox21/ToxCast ~700 HTS Biological Pathways Assays

National Institute of Environmental Health Sciences (NIEHS) / National Toxicology Program (NTP)

National Center for Advancing Translational Sciences (NCATS)

U.S. Food and Drug Administration (FDA)

National Center for Computational Toxicology (EPA)

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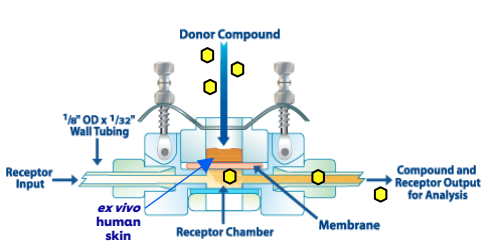
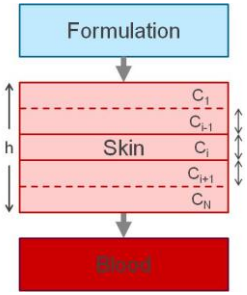
NGRA: The assessment is exposure-led

- **Route of exposure**
- **Consumer use (Habits &Practices)**
- **Applied dose (external concentration)**

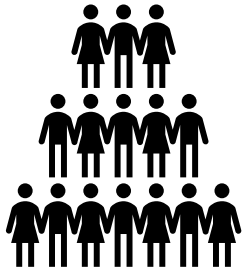
ADME parameters



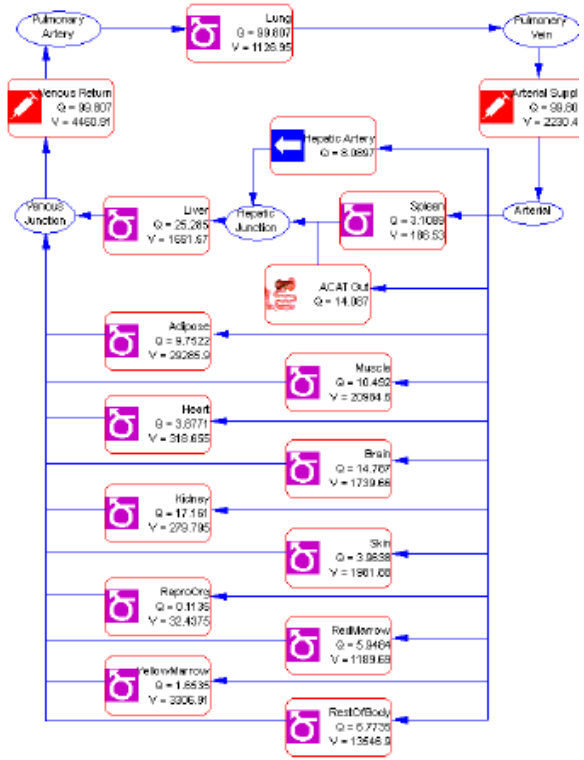


- **Skin penetration**
- **Phys-chem properties**
- **Hepatic clearance**
- **Fraction unbound**
- **blood:plasma ratio**

Uncertainty analysis- Population simulation



Physiologically-based kinetic (PBK) modelling – Internal concentration (plasma, urine, organ-level)



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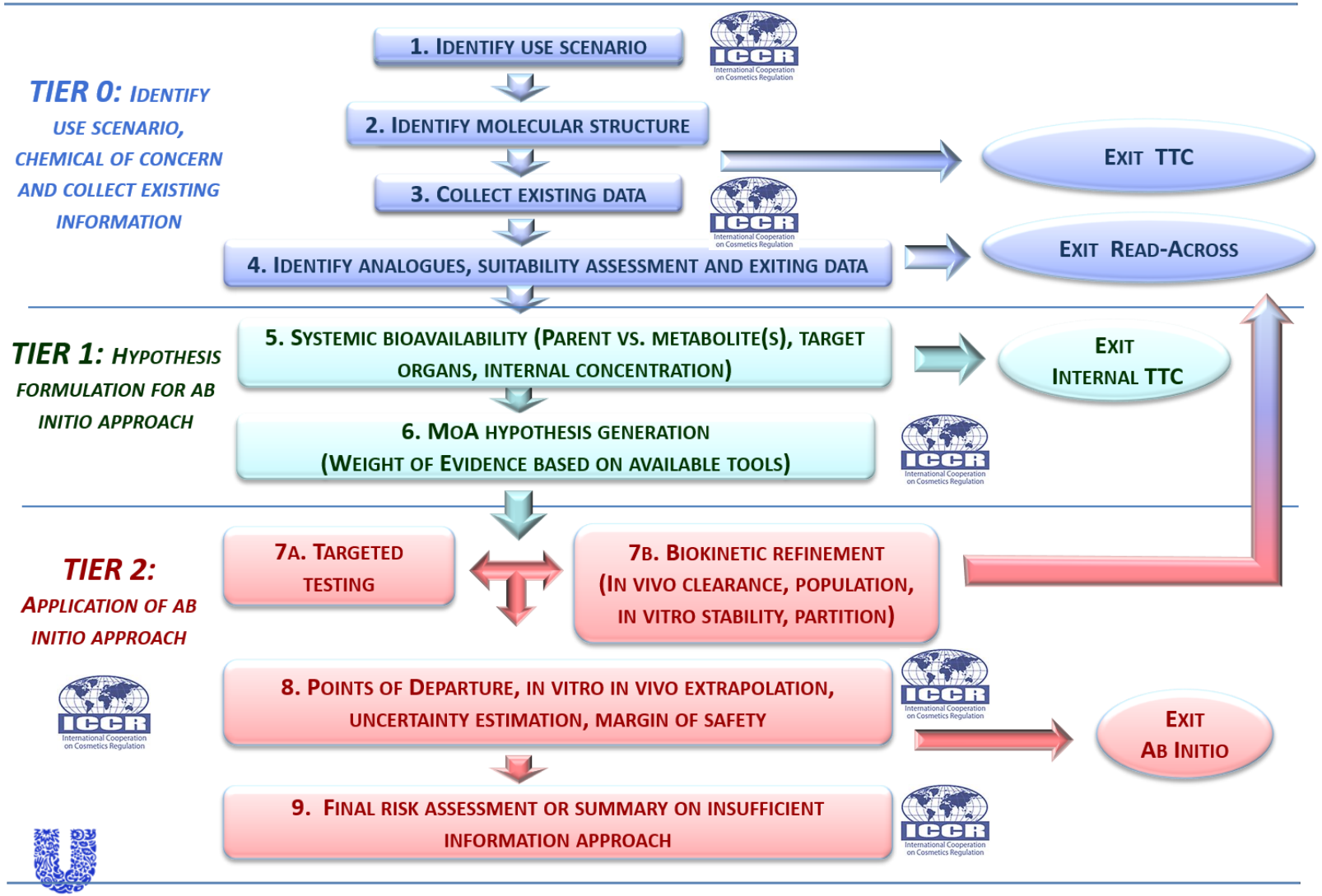
NGRA: The assessment is hypothesis driven & should be conducted Using a tiered and iterative approach



SEURAT-1

Continue through tiers until enough information to make a decision: assessment may be complete at any tier

Berggren *et al.*, (2017) *Computational Toxicology* 4: 31-44. https://doi.org/10.1016/j.comtox.2017.10.001

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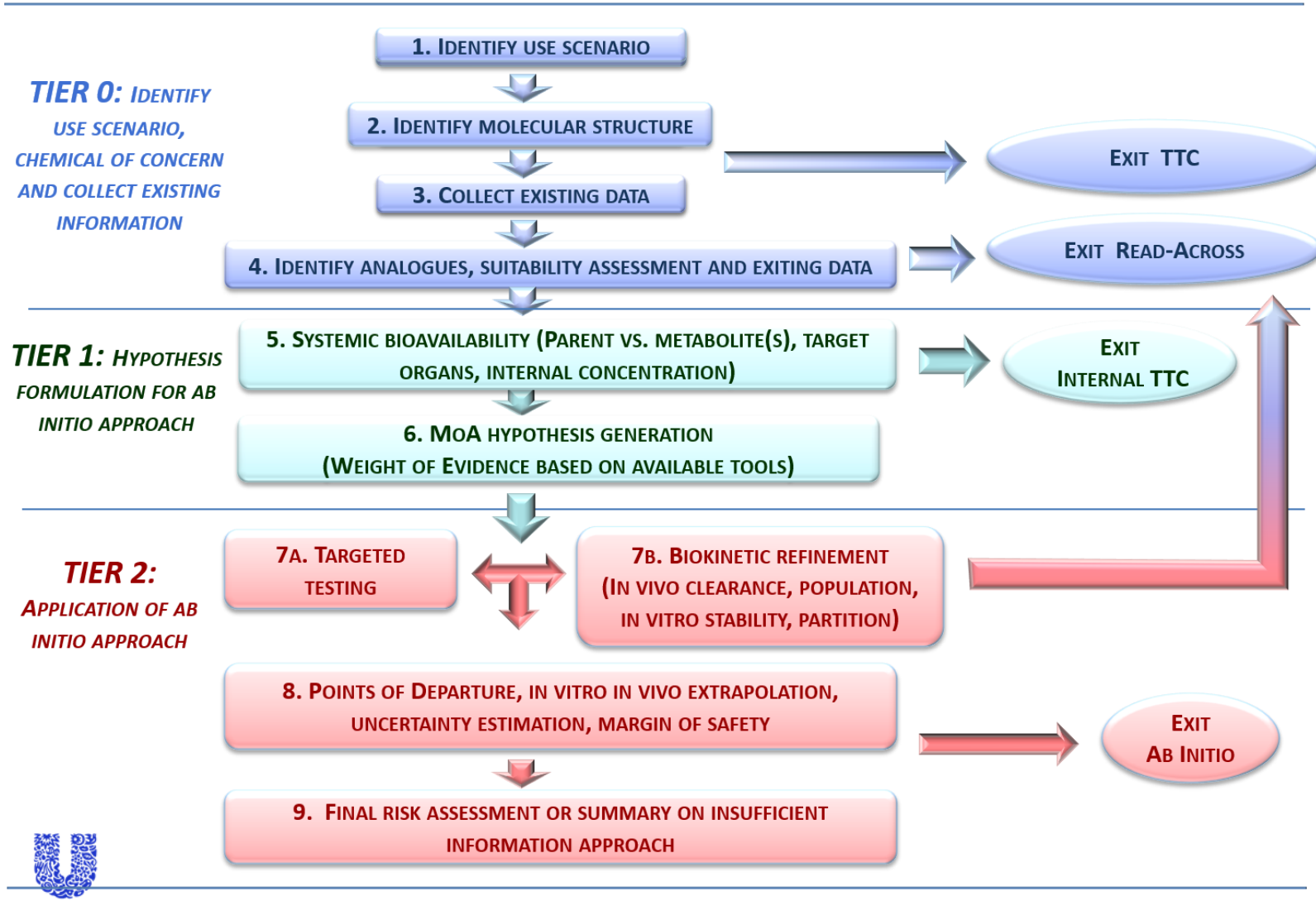
NGRA: Using robust and relevant methods and strategies



Read across

Exposure-based waiving

In silico tools

Metabolism and metabolite identification

Physiologically-based kinetic modelling

In chemico assays

'Omics

Reporter gene assays

In vitro pharmacological profiling

3D culture systems

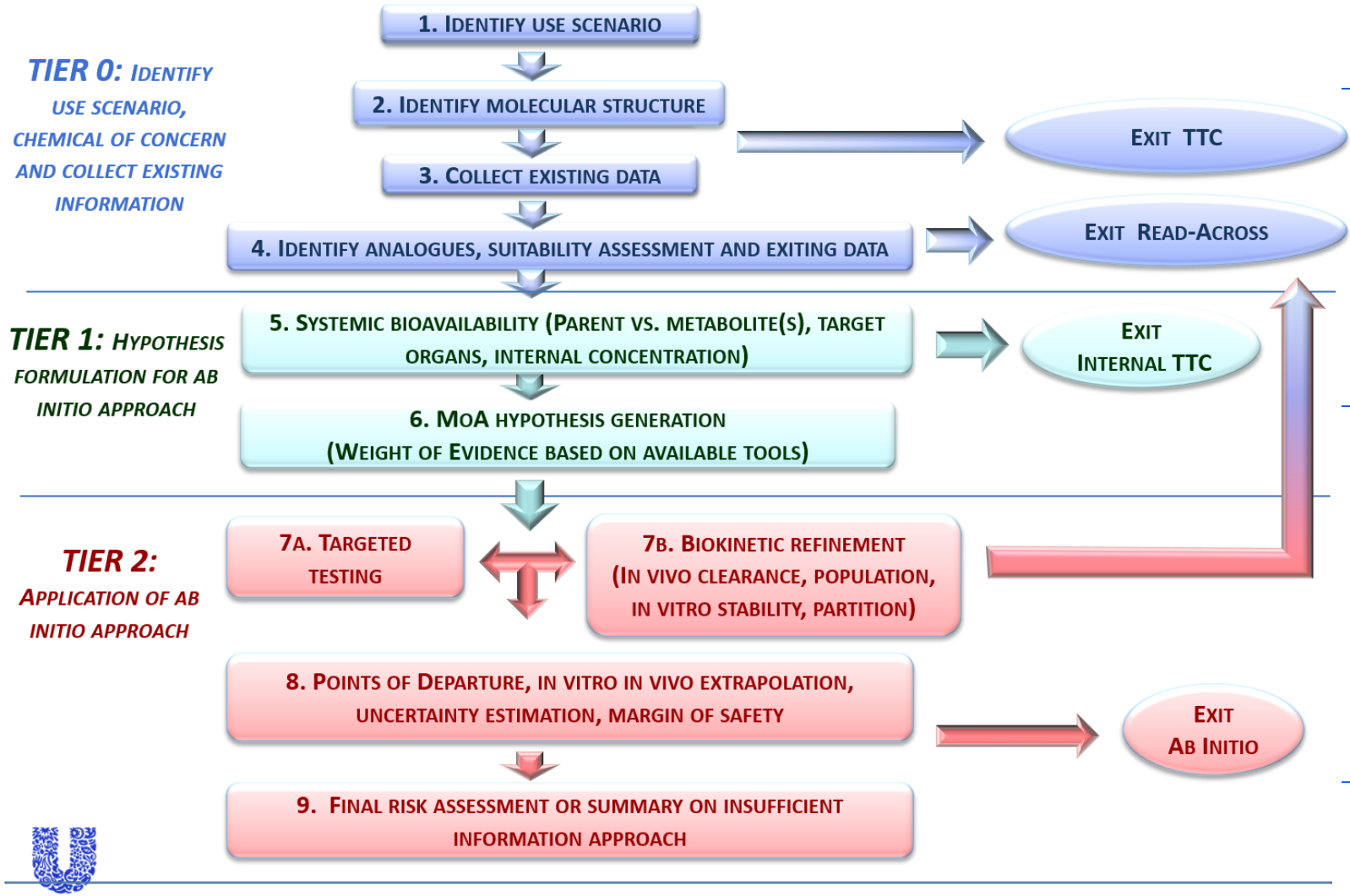
Organ-on-chip

Zebrafish larva assays

Pathways modelling

Human studies

NGRA: Using robust and relevant methods and strategies



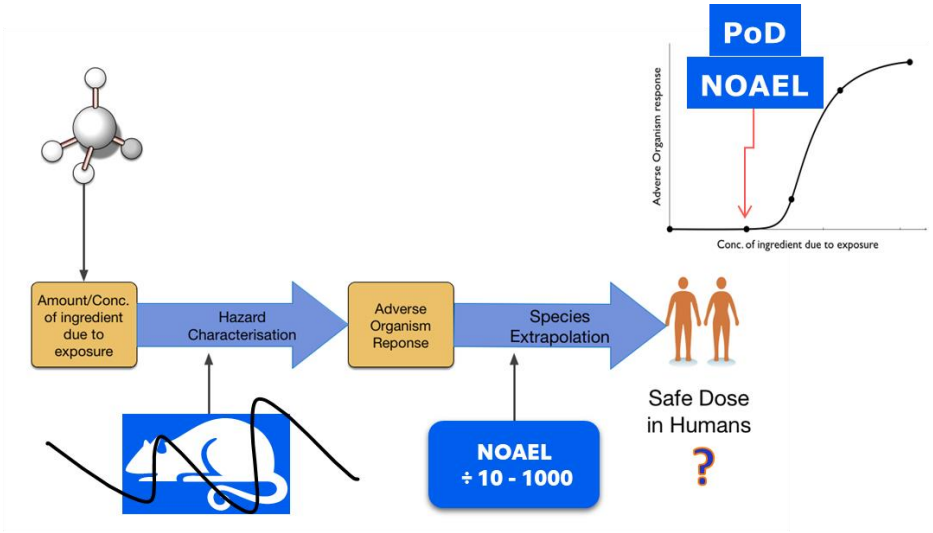
Readiness judged by ICCR in 2018:
(ICCR IS JWG Part 2 FINAL (iccr-cosmetics.org)

- Read across
- Exposure-based waiving
- *In silico* tools
- Metabolism and metabolite identification
- Physiologically-based kinetic modelling
- *In chemico* assays
- 'Omics
- Reporter gene assays
- *In vitro* pharmacological profiling
- 3D culture systems
- Organ-on-chip
- Zebrafish larva assays
- Pathway modelling
- Human studies

Note - not universally considered a non-animal approach

NGRA: The assessment is designed to prevent harm
Focus on protection

- Non-specific endpoints from in vivo toxicological studies data are often used to derive points of departure (POD) (e.g. no-observed-effect-level or no-observed adverse effect level (NO(A)EL))
- Uncertainty or safety assessment factors are applied to POD to calculate recommended exposure levels that are broadly protective but not necessarily target-specific.



Are non-animal safety assessments even possible for systemic toxicity?

Many possible adversities…ADME considerations…Homeostasis

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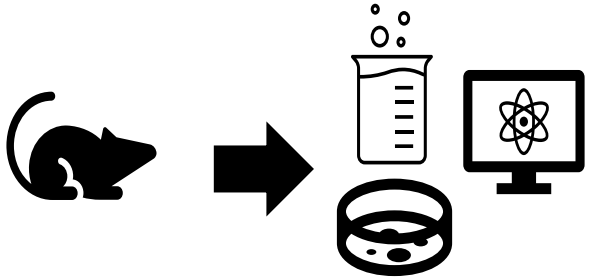
Yes… but it requires a different way of thinking about the problem



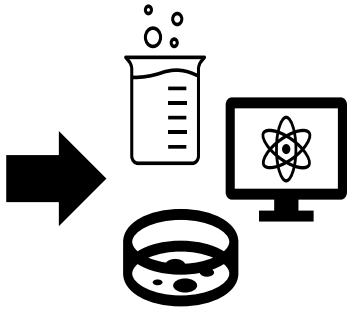
Non-animal NAMs strategies for 1-2-1 replacement – prediction of animal outcome

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



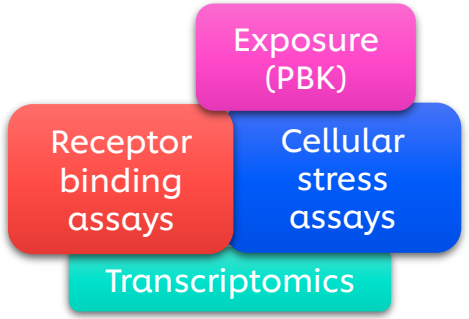
Development of battery of assays aligned to AOPs

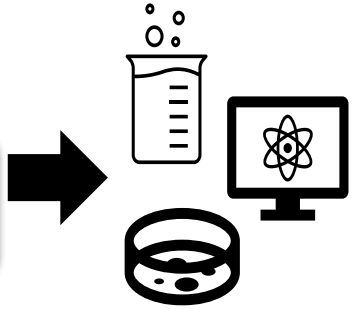
Not feasible as a tier 1 approach

~ 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

Protection 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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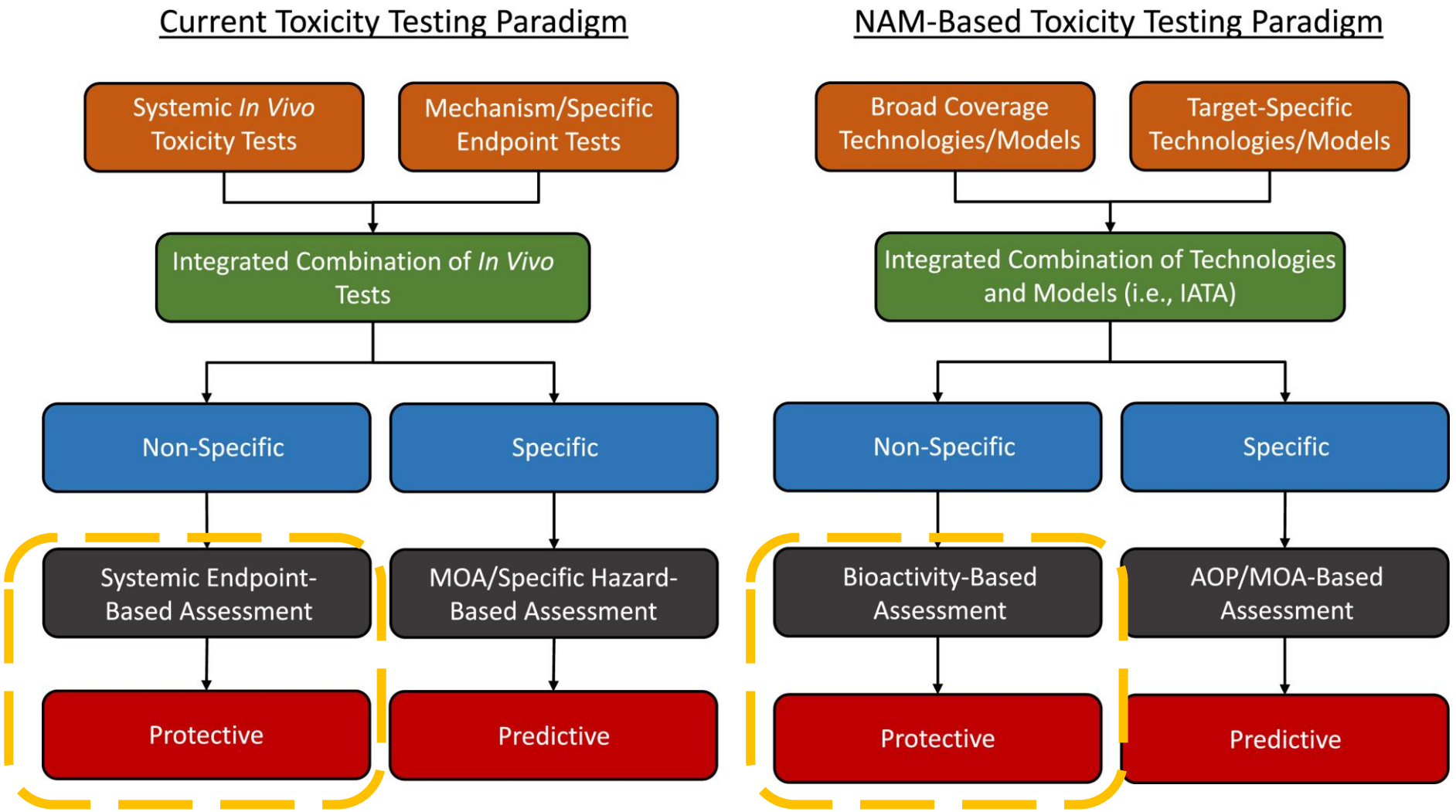
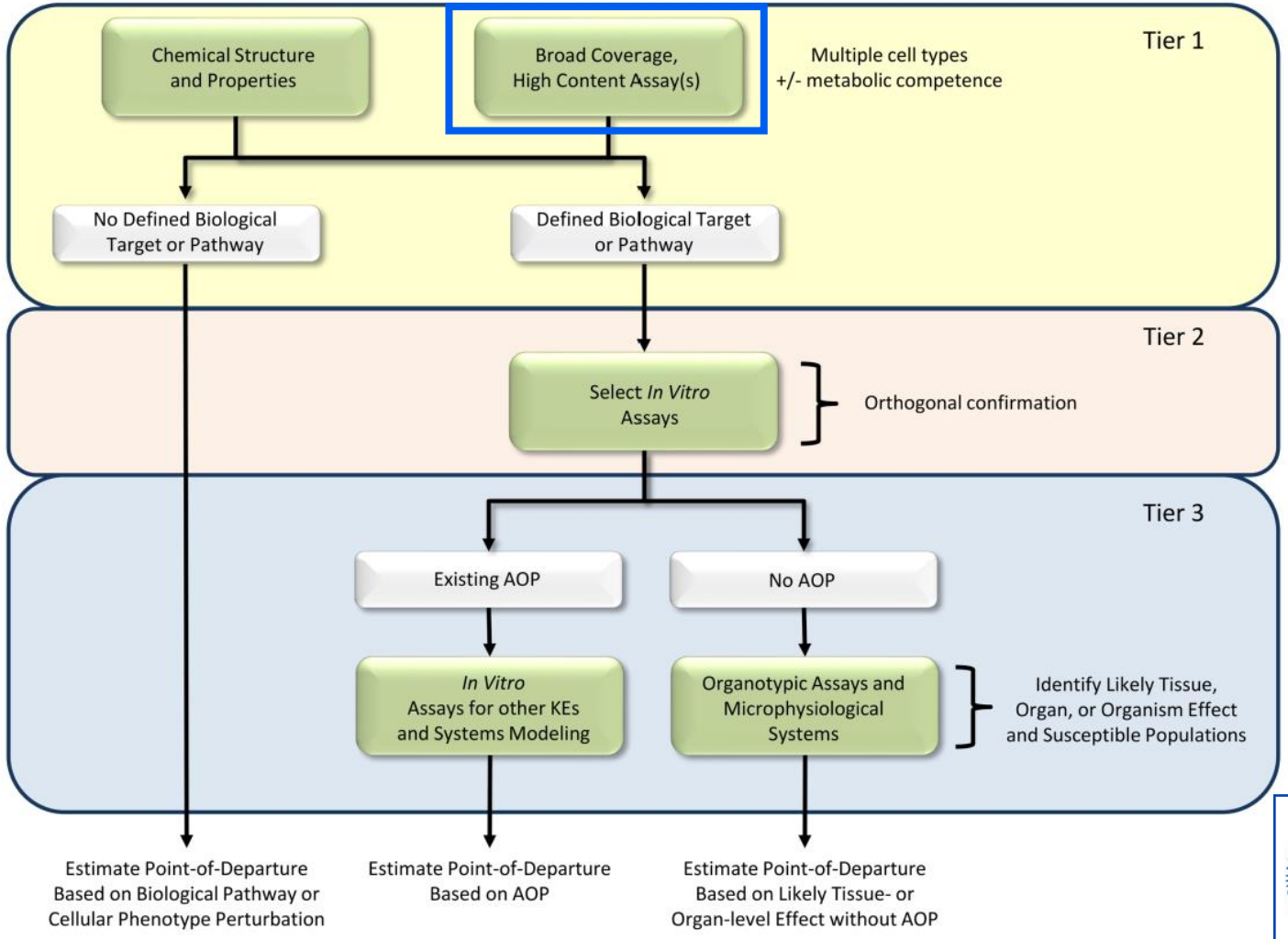
Current toxicity paradigm & NGRA both designed to prevent harm



Browne et al., 2024 Reg Tox Pharm https://doi.org/10.1016/j.yrtph.2024.105579

Example from the US EPA framework for deriving protective PoDs

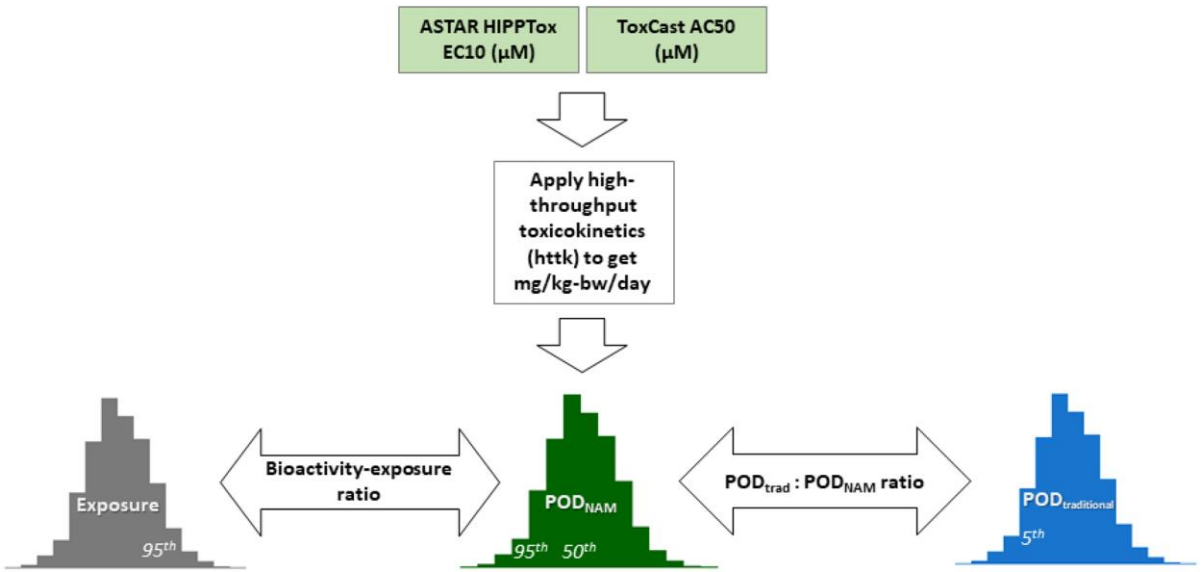
High-throughput transcriptomics (HTTr)[1] and High-throughput phenotypic profiling(HTTP)[2] developed to increase biological coverage



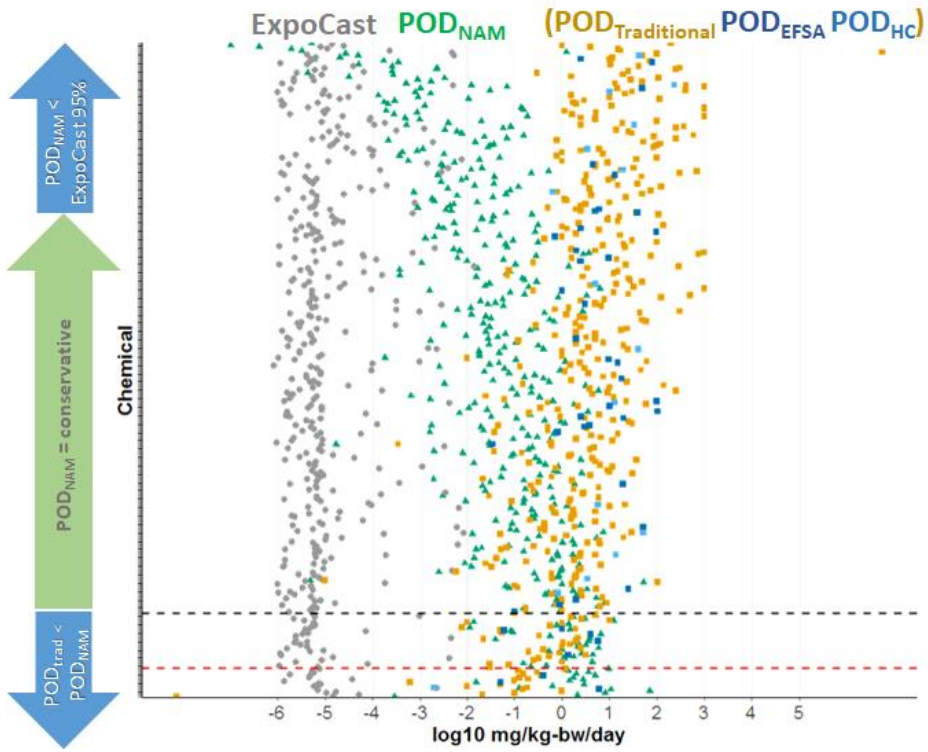
1. Harrill J et al 2019. Considerations for strategic use of high-throughput transcriptomics chemical screening data in regulatory decisions. Current Opinion in Toxicology 15, 64-75.
2. Nyffeler J et al 2019. Bioactivity screening of environmental chemicals using imaging-based high-throughput phenotypic profiling. *Toxicol Appl Pharmacol*. 2020;389:114876.

Russell S Thomas et al., 2019. The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency. Tox Sci 169(2):317-332.

Case Study Demonstrating Application of Bioactivity as a Protective POD

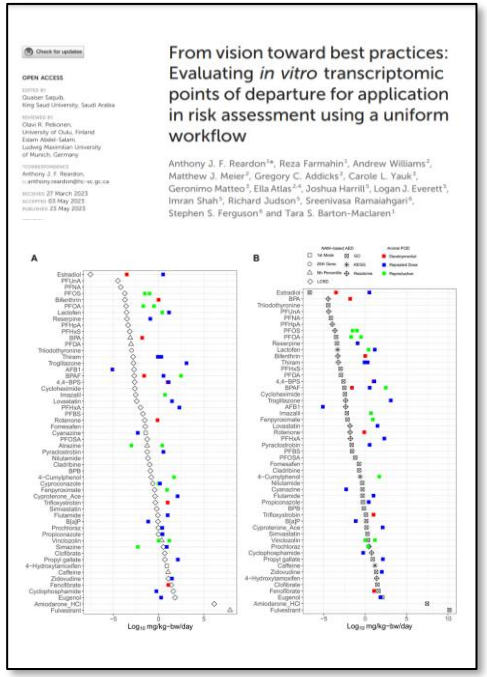


[Paul-Friedman et al., 2020]

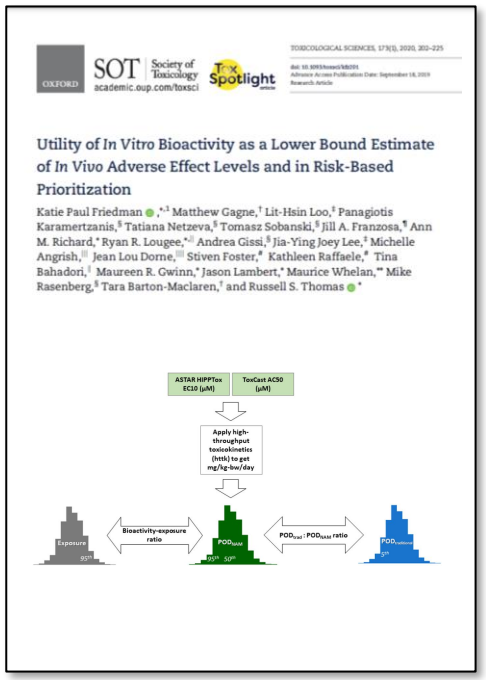


- For 89% of the chemicals NAM PoD was more conservative than the traditional POD.
- Bioactivity:exposure ratios (BERs) approach useful for accelerate screening and assessment using NAMs for hazard and exposure.

Examples of ongoing or completed case studies for NAM/NGRA BER based risk assessment or prioritisation

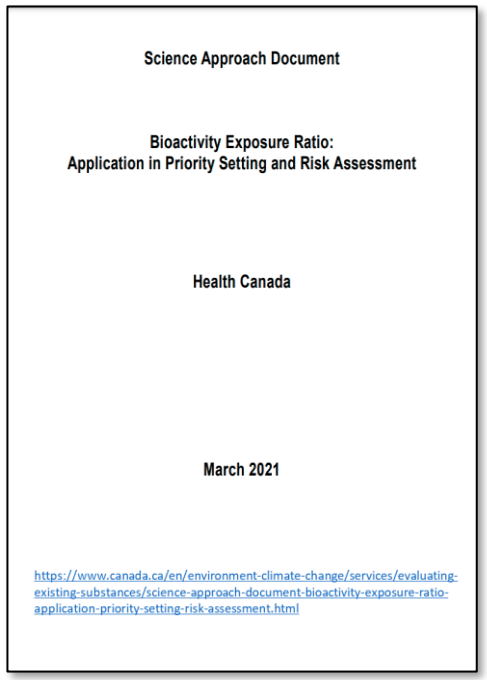

From vision toward best practices: Evaluating *in vitro* transcriptomic points of departure for application in risk assessment using a uniform workflow

Anthony J. F. Reardon[1*], Reza Farmahin[1], Andrew Williams[2], Matthew J. Meier[2], Gregory C. Addicks[2], Carole L. Yauk[3], Geronimo Matteo[2], Ella Atlas[2,4], Joshua Harrill[5], Logan J. Everett[3], Imran Shah[5], Richard Judson[5], Sreenivasa Ramaiahgari[6], Stephen S. Ferguson[6] and Tara S. Barton-Maclaren[1]


SOT Society of Toxicology academic.oup.com/toxsci Tox Spotlight

Utility of *In Vitro* Bioactivity as a Lower Bound Estimate of *In Vivo* Adverse Effect Levels and in Risk-Based Prioritization

Katie Paul Friedman, [*,1] Matthew Gagne,[†] Lit-Hsin Loo,[‡] Panagiotis Karamertzanis,[§] Tatiana Netzeva,[§] Tomasz Sobanski,[§] Jill A. Franzosa,[¶] Ann M. Richard,[*] Ryan R. Lougee,[*,∥] Andrea Gissi,[§] Jia-Ying Joey Lee,[‡] Michelle Angrish,[⊥] Jean Lou Dorne,[#] Stiven Foster,[#] Kathleen Raffaele,[#] Tina Bahadori,[⊥] Maureen R. Gwinn,[*] Jason Lambert,[*] Maurice Whelan,[**] Mike Rasenberg,[§] Tara Barton-Maclaren,[†] and Russell S. Thomas [*]

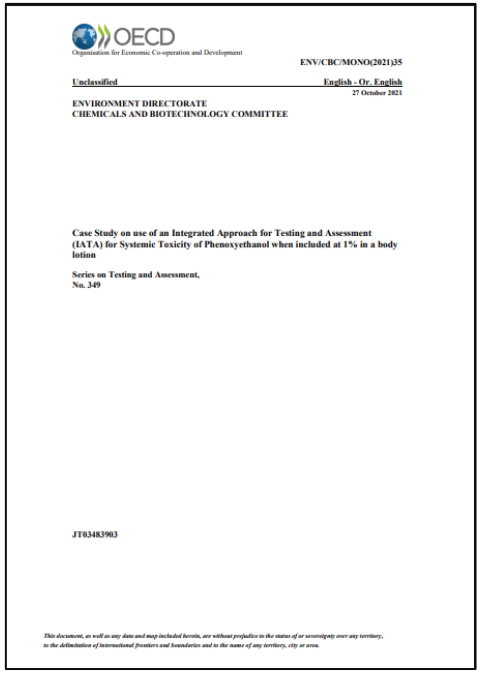

Science Approach Document

Bioactivity Exposure Ratio:
Application in Priority Setting and Risk Assessment

Health Canada

March 2021

https://www.canada.ca/en/environment-climate-change/services/evaluating-existing-substances/science-approach-document-bioactivity-exposure-ratio-application-priority-setting-risk-assessment.html


OECD
Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2021)35

Unclassified — English - Or. English
27 October 2021

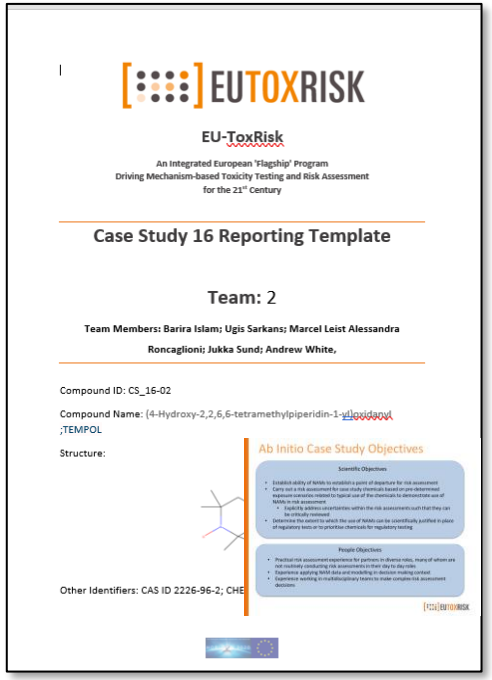
ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

Case Study on use of an Integrated Approach for Testing and Assessment (IATA) for Systemic Toxicity of Phenoxyethanol when included at 1% in a body lotion

Series on Testing and Assessment,
No. 349

JT03483903

This document, as well as any data and map included herein, are without prejudice to the status of or sovereignty over any territory, to the delimitation of international frontiers and boundaries and to the name of any territory, city or area.


EUTOXRISK

EU-ToxRisk

An Integrated European 'Flagship' Program
Driving Mechanism-based Toxicity Testing and Risk Assessment
for the 21st Century

Case Study 16 Reporting Template

Team: 2

Team Members: Barira Islam; Ugis Sarkans; Marcel Leist Alessandra Roncaglioni; Jukka Sund; Andrew White,

Compound ID: CS_16-02

Compound Name: (4-Hydroxy-2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl ;TEMPOL

Structure:

Ab Initio Case Study Objectives

Other Identifiers: CAS ID 2226-96-2; CHE

Health Canada

United States Environmental Protection Agency

United States Environmental Protection Agency

Health Canada

Cosmetics Europe the personal care association

Cosmetics Europe LRSS

RISK HUNT3R

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Unilever development of a systemic toolbox

A NAMs/NGRA Tiered Framework Approach: The overall goal is a human safety risk assessment

Tier 0: Problem Formulation

- Characterise the chemical
- Characterise the consumer exposure scenario
- Collate all available information (literature mining)

→ **Exit** *if safety decision can be made*

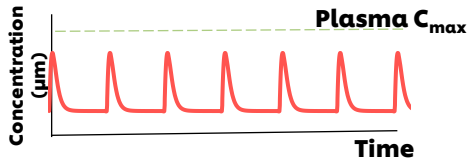
- Use of in silico tools
- Exposure-based waiving (TTC)
- Read Across

→ **Exit** *if safety decision can be made*

Decision cannot be made

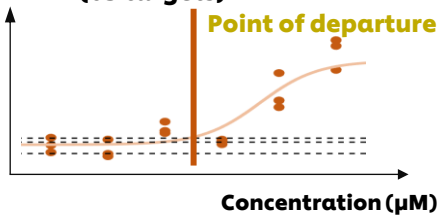
Tier 1: Systemic-safety toolbox

Use of PBK models to estimate internal exposure levels (C_{max})



Generation of bioactivity data:

- Cell Stress Panel
- HTTr (MCF7, HepaRG, HepG2)
- IPP (63 targets)



→ **Calculate BER and compare to BER thresholds**

→ **Weight of evidence assessment with Tier 0 and Tier 1 information**

→ **Exit** *if safety decision can be made*

Decision cannot be made

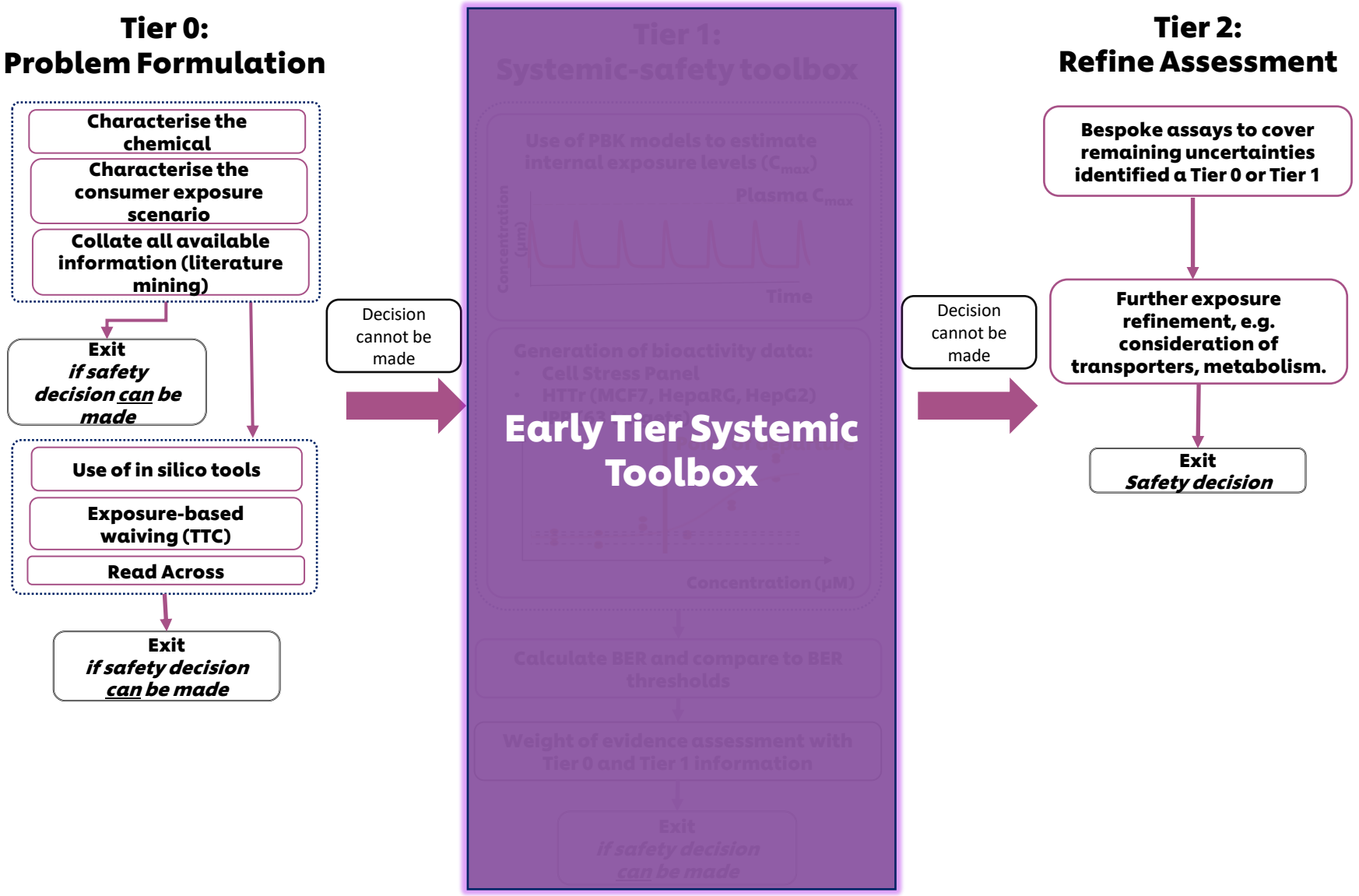
Tier 2: Refine Assessment

Bespoke assays to cover remaining uncertainties identified a Tier 0 or Tier 1

→ **Further exposure refinement, e.g. consideration of transporters, metabolism.**

→ **Exit** *Safety decision*

A NAMs/NGRA Tiered Framework Approach: The overall goal is a human safety risk assessment



Evaluation/"Validation" of an Early Tier Toolbox for Systemic Safety

AIM: Use NAMs to ensure the protection of consumers: can the approach be used to confidently identify high/low risk chemical exposure scenarios?

1. **Define the toolbox components** Choose and evaluate a set of NAMs covering exposure modelling and bioactivity investigations
2. **Select test chemicals** Choose as many as practicable to maximise coverage of different chemistries and biological effects/toxicity
3. **Set performance criteria** Define the 'truth' that the performance of the toolbox will be compared to

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Evaluation split into 2 stages: Pilot and extended evaluation

Pilot study (Middleton et al., 2022)[1]

Define what the toolbox contains (which NAMs) and the workflow & protocols

Set performance criteria

10 chemicals selected by experts
24 exposure scenarios

Define prototype decision model for determining protective BER threshold

Extended evaluation (Cable et al., 2025)[2]

Repeat protocols/workflow as established in Middleton et al. 2022

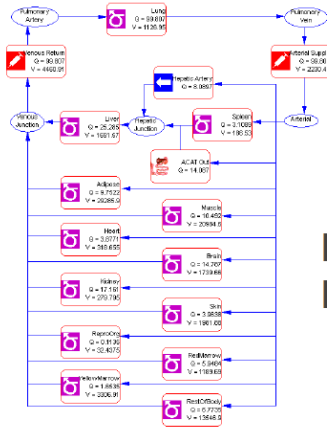
38 chemicals selected semi-randomly
70 exposure scenarios

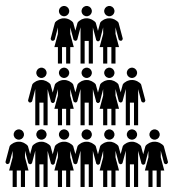
Apply the BER threshold

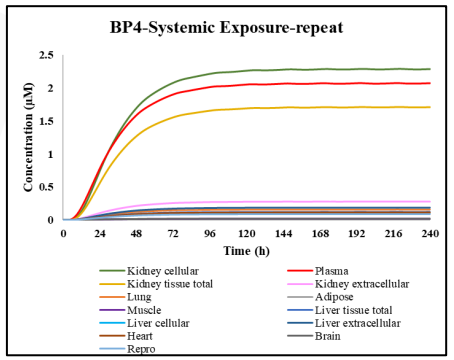
1. Middleton et al. (2022) (https://doi.org/10.1093/toxsci/kfac068)
2. Cable et al., 2025: https://doi.org/10.1093/toxsci/kfae159

Our Key NAMs

Internal exposure - PBK modelling

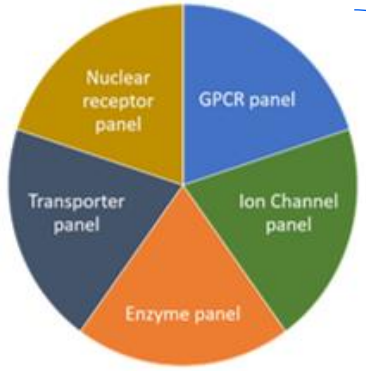






Moxon TE et al., 2020. Toxicology In Vitro, 63, 104746

In vitro pharmacological profiling



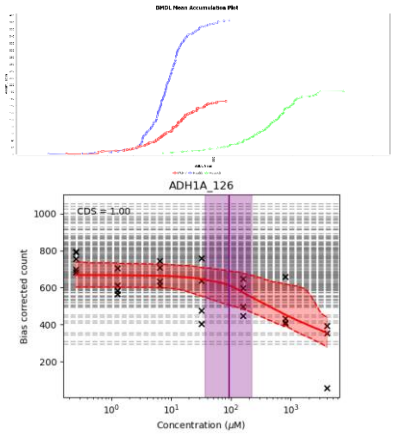
~79 targets

eurofins | Cerep

Bowes et al. 2012. Nat Rev Drug Discov 11(12): 909-22

High-Throughput transcriptomics (HTTr)

- TempO-seq technology – full gene panel
- 24hr exposure
- 7 concentrations
- Various cell models (e.g. HepG2, MCF7, HepaRG)
- Dose-response analysis using BMDExpress2 and BIFROST model



Reynolds et al. 2020. Comp Tox 16: 100138
Baltazar et al. 2020. Toxicol Sci 176(1): 236–252

Cell stress panel (CSP)

- 36 biomarkers covering 10 cell stress pathways
- HepG2
- 24hr exposure
- 8 concentrations
- Dose-response analysis using BIFROST model

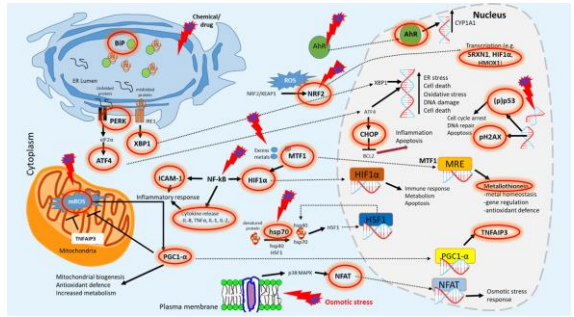


Image kindly provided by Paul Walker (Cyprotex)

Hatherell et al. 2020. Toxicol Sci 176(1): 11-33

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Defining the toolbox components

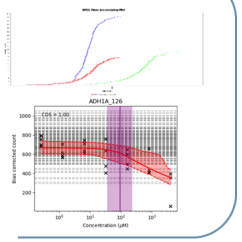
Pilot study (Middleton et al., 2022)

Point of Departure determination from Bioactivity assays

Non-specific effects

High-Throughput transcriptomics (HTTr)

- TempO-seq technology – full gene panel
- 24hr exposure
- 7 concentrations
- Various cell models (e.g. HepG2, MCF7, HepaRG)
- Dose-response analysis using BMDExpress2 and BIFROST model



Reynolds et al. 2020. Comp Tox 16: 100138
Baltazar et al. 2020. Toxicol Sci 176(1): 236–252

Cell stress panel (CSP)

- 36 biomarkers covering 10 cell stress pathways
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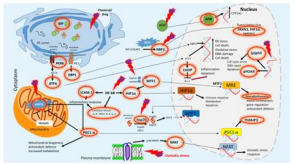
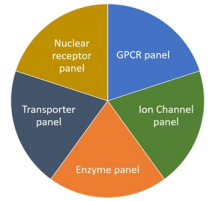


Image kindly provided by Paul Walker (Cyprotex)

Hatherell et al. 2020. Toxicol Sci 176(1): 11-33

Specific effects

In vitro pharmacological profiling



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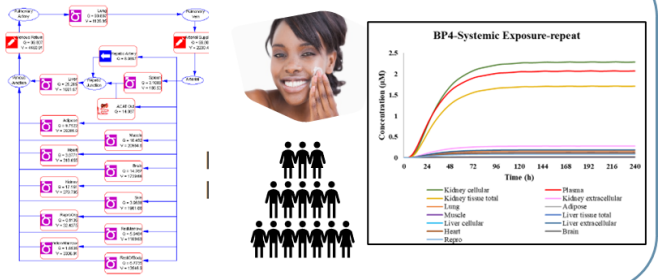
Internal exposure - PBK modelling

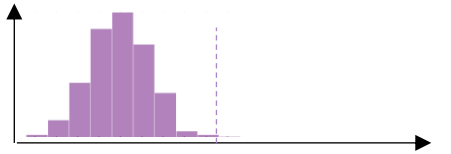


Moxon TH et al., 2020. Toxicology In Vitro, 63, 104746

Plasma C_{max} estimate → **C_{max} Error Distribution model (CMED)**

(Bayesian model)

Bioactivity Exposure Ratio Distribution



Bioactivity Exposure Ratio (log10)

Unilever

Standardisation of experimental design & computational pipelines

Pilot study (Middleton et al., 2022)

Point of Departure determination

Non-specific effects

High-Throughput transcriptomics (HTTr)

- TempO-seq technology – full gene panel
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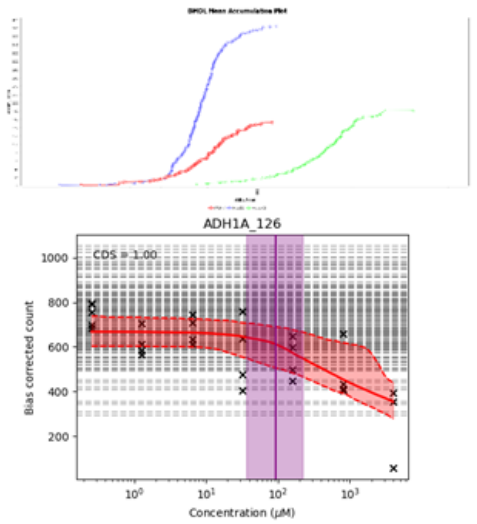

Reynolds et al. 2020. Comp Tox 16: 100138
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PODs obtained from Transcriptomics for each cell line, MCF7, HepG2, HepaRG 2D:

- **The minimum pathway BMDL** from the transcriptomics platform estimated using BMDExpress.
- **The global POD** from the transcriptomics platform estimated using BIFROST. The global POD represents an estimate of the minimum effect concentration across all genes. The method quantifies uncertainty in the POD as a probability distribution for each gene.

Unilever

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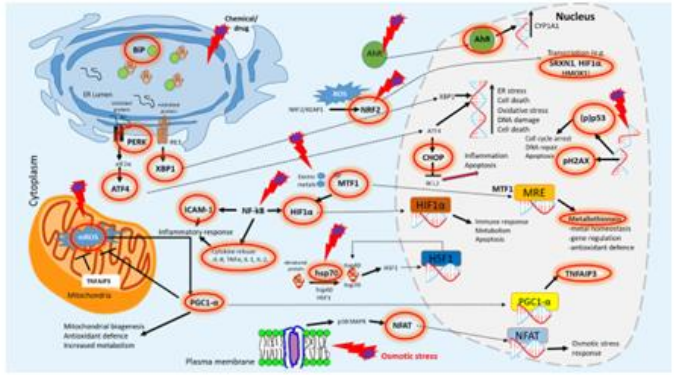


Image kindly provided by Paul Walker (Cyprotex)

Hatherell et al. 2020. Toxicol Sci 176(1): 11-33

- **The CSP global POD**, as estimated using BIFROST (i.e. minimum across all 36 biomarkers)
- The global POD represents an estimate of the minimum effect concentration across all biomarkers. The method quantifies uncertainty in the POD as a probability distribution for each biomarker.

Unilever

Standardisation of experimental design & computational pipelines

Pilot study (Middleton et al., 2022)

Point of Departure determination

Specific effects

In vitro pharmacological profiling

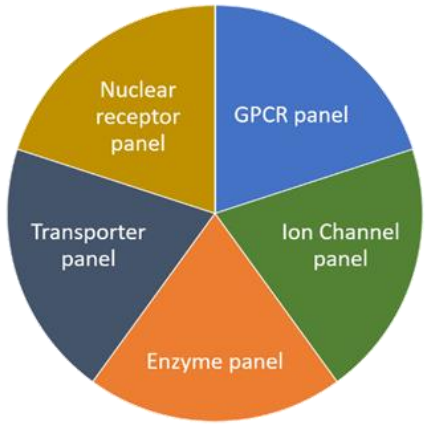

PERSPECTIVES

A GUIDE TO DRUG DISCOVERY — OPINION

Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

Joanne Bowes, Andrew J. Brown, Jacques Hamon, Wolfgang Jarolimek, Arun Sridhar, Gareth Waldron and Steven Whitebread

Abstract | In vitro pharmacological profiling is increasingly being used earlier in the drug discovery process to identify undesirable off-target activity profiles that could hinder or halt the development of candidate drugs or even lead to market withdrawal if discovered after a drug is approved. Here, for the first time, the rationale, strategies and methodologies for *in vitro* pharmacological profiling at four major pharmaceutical companies (AstraZeneca, GlaxoSmithKline, Novartis and Pfizer) are presented and illustrated with examples of their impact on the drug discovery process. We hope that this will enable other companies and academic institutions to benefit from this knowledge and consider joining us in our collaborative knowledge sharing.



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- Panel developed by the pharmaceutical industry and used during early drug discovery to predict, assess and minimise/avoid risk of potential off-target adverse drug reactions.
- Initial panel of 44 targets identified to be related to adverse health outcomes
- Extended to 63 targets to include extra nuclear receptors
- Experiment in 2 phases:
- Screening at a fixed concentration (10 or 100 µM)
- **Dose-response assays on positive hits to identify a point of departure (PoD) expressed as an IC50 value**

1. Bowes J et al 2012. Nat Rev Drug Discov;11(12):909-22.
2. Lynch JJ et al., 2017 Pharmacol Toxicol Methods;87:108-126.
3. Smit IA et al., 2021 Chem Res Toxicol;34(2):365-384.
4. Letswaart R et al., 2020 EBioMedicine;57:102837

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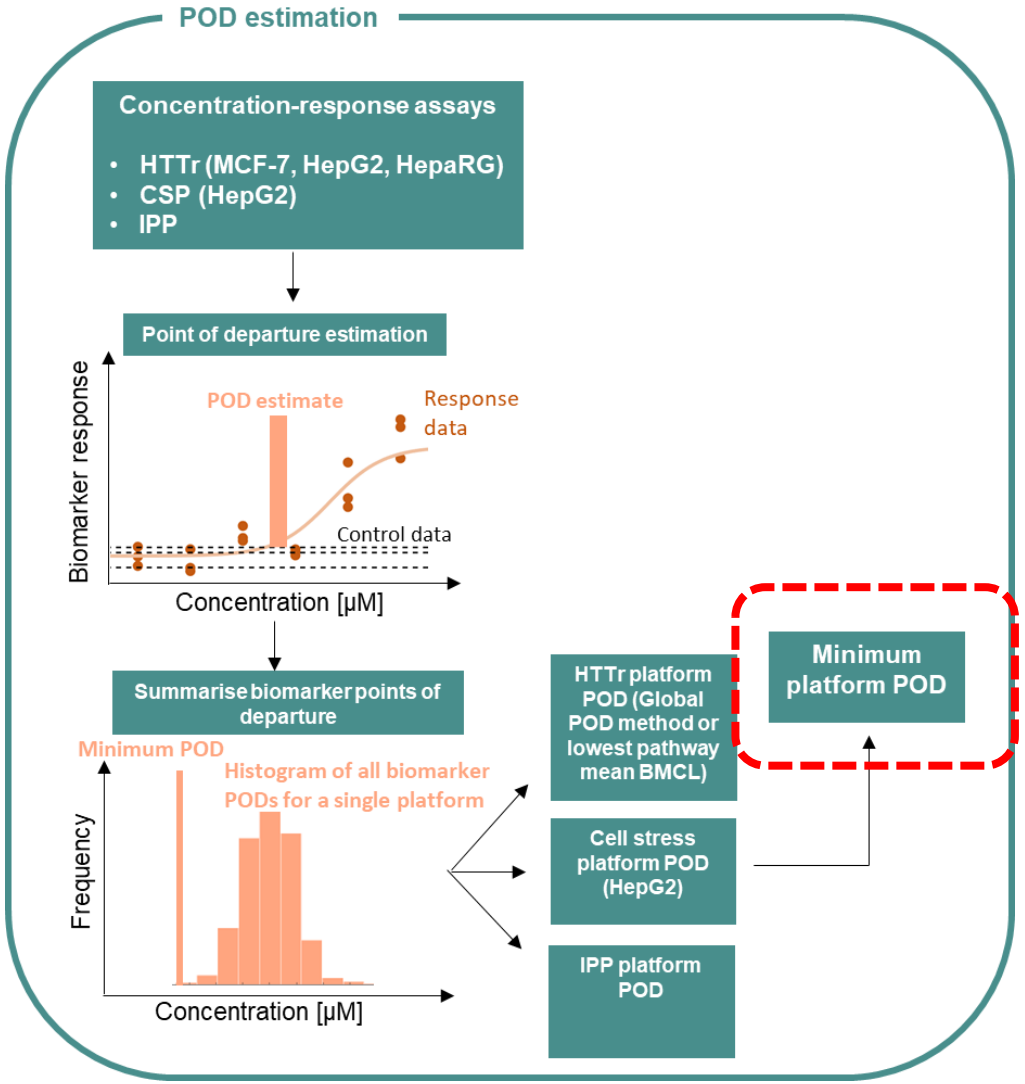
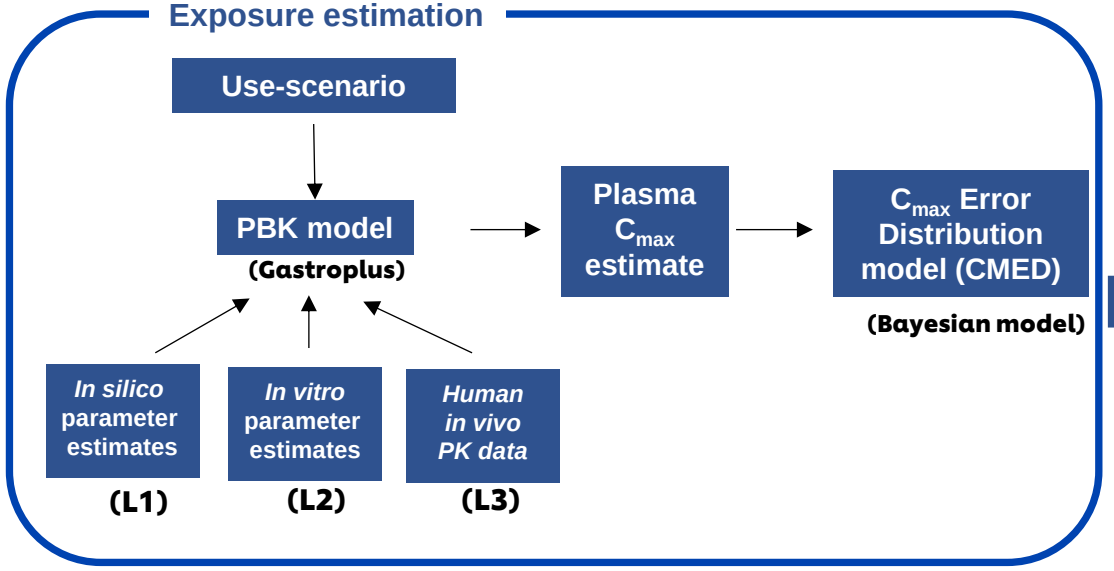
Estimating PODs from bioactivity platforms- Minimum POD is selected for calculating a BER

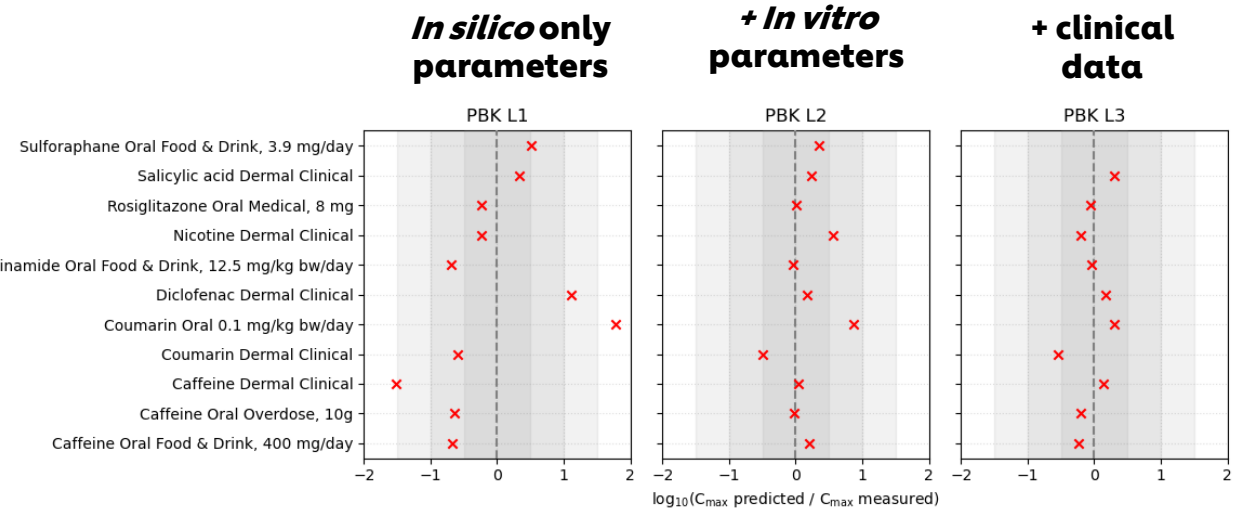
Pilot study (Middleton et al., 2022)



Estimation of plasma C_{max} using Physiologically-based kinetic modelling: workflow & uncertainty analysis

Pilot study (Middleton et al., 2022)





- The PBK prediction error decreases as we go 'up' parameterisation levels
- Developed a Bayesian statistical model to quantify the error for a novel chemical
- **<u>Output:</u> Plasma C_{max} distribution at each PBK level**

Unilever

Defining the toolbox components

Pilot study (Middleton et al., 2022)

Point of Departure determination

Non-specific effects

High-Throughput transcriptomics (HTTr)

- TempO-seq technology – full gene panel
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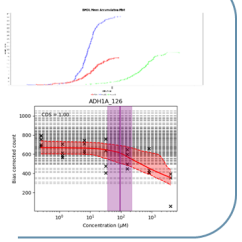

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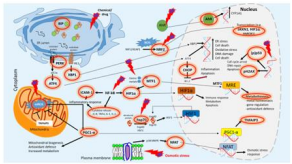

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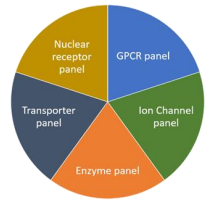

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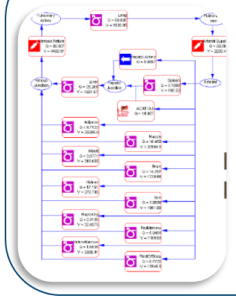
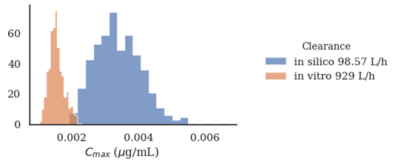
PBK Modelling



Face Cream



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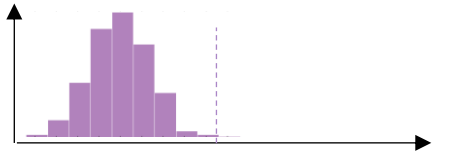

Unilever

Set performance criteria

Pilot study (Middleton et al., 2022)

- Assuming the current risk assessments are protective for human health:
  - The performance of the NAM toolbox is assessed against historical safety decisions
  - Benchmark chemical-exposure scenarios with known outcomes, low and high risk to define a safe BER threshold

What we are trying to test: Are the decisions made with a Tier 1 toolbox equivalent or better than the decisions we have been making with animal data?

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

Unilever

Set performance criteria for evaluating the protectiveness and utility of the toolbox

Pilot study (Middleton et al., 2022)

Benchmarking using chemical-exposure scenarios

- Chemicals with well-defined human exposures
- Traditional safety assessment available (e.g. regulatory opinions)
- Risk benchmarked to acceptability in a consumer product context

Protectiveness

How many of the high risk exposure scenarios are identified as uncertain/high risk
(i.e. BER < threshold)

Utility

How many of the low risk scenarios are identified as low risk at this early tier stage in a risk assessment framework
(i.e. BER > threshold)

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Select test chemicals with known human exposure and associated risk assessments

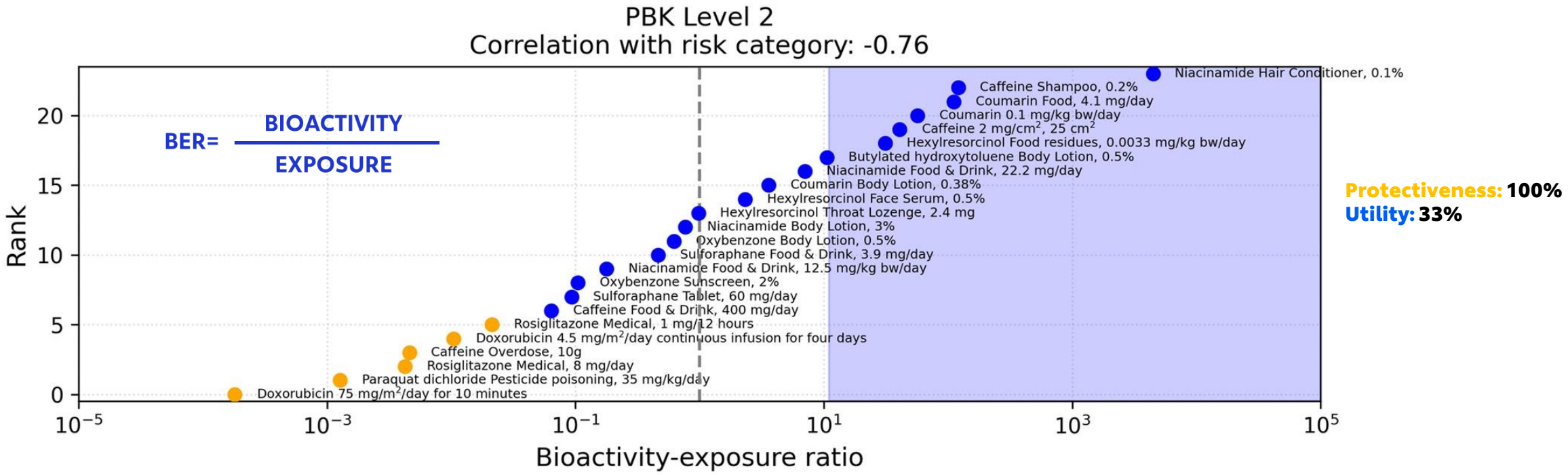
Pilot study (Middleton et al., 2022)

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

Unilever

NAM Systemic toolbox 100% protective for high-risk chemical exposure scenarios

Pilot study (Middleton et al., 2022)



Protectiveness: **100%**
Utility: **33%**

Chemical-exposure scenarios with a BER point estimate outside the blue-shaded region would be identified as “uncertain” risk under this decision model. The grey-dashed line corresponds to BER = 1. Blue shaded region BER> 11 corresponding to threshold BER for PBK level 2 above which an exposure would be considered low risk. Blue circles: low risk chemical-exposure scenario; Yellow circles: high risk chemical-exposure scenario

Threshold values of the BER point estimates for determining whether an exposure is low risk are dependent on the confidence on the PBK model

Pilot study (Middleton et al., 2022)

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

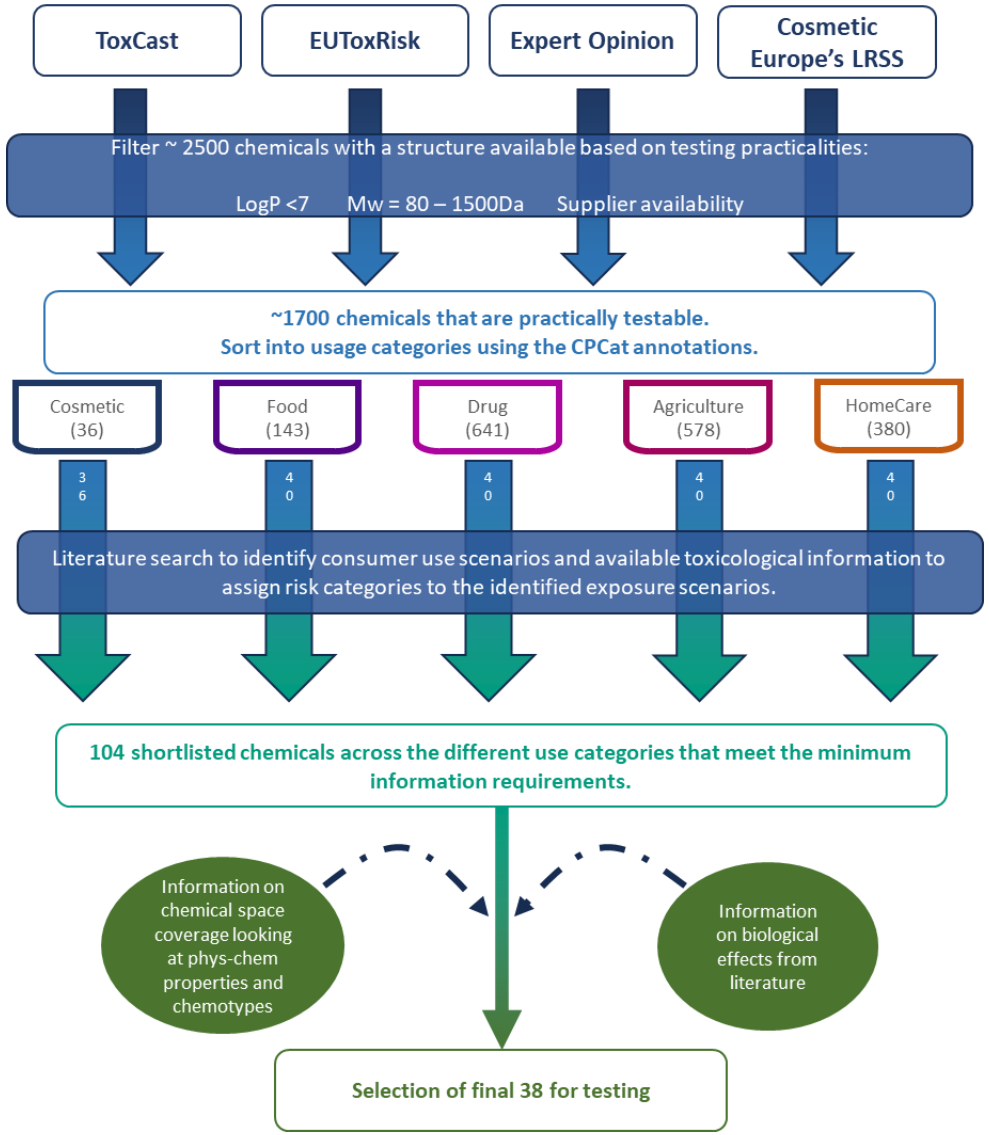
Are these thresholds still protective if we increase the number and diversity of chemicals?

Extended evaluation (Cable et al., in preparation)

Unilever

Semi-random selection of the 38 chemicals covering multiple use categories and chemistry

Extended evaluation (Cable et al., 2025)



Semi-random selection of the 38 chemicals covering multiple use categories and chemistry

Extended evaluation (Cable et al., 2025)

38 test chemicals

- 9 cosmetic ingredients, 21 drugs, 3 food additives, 5 agricultural chemicals, 1 industrial chemical

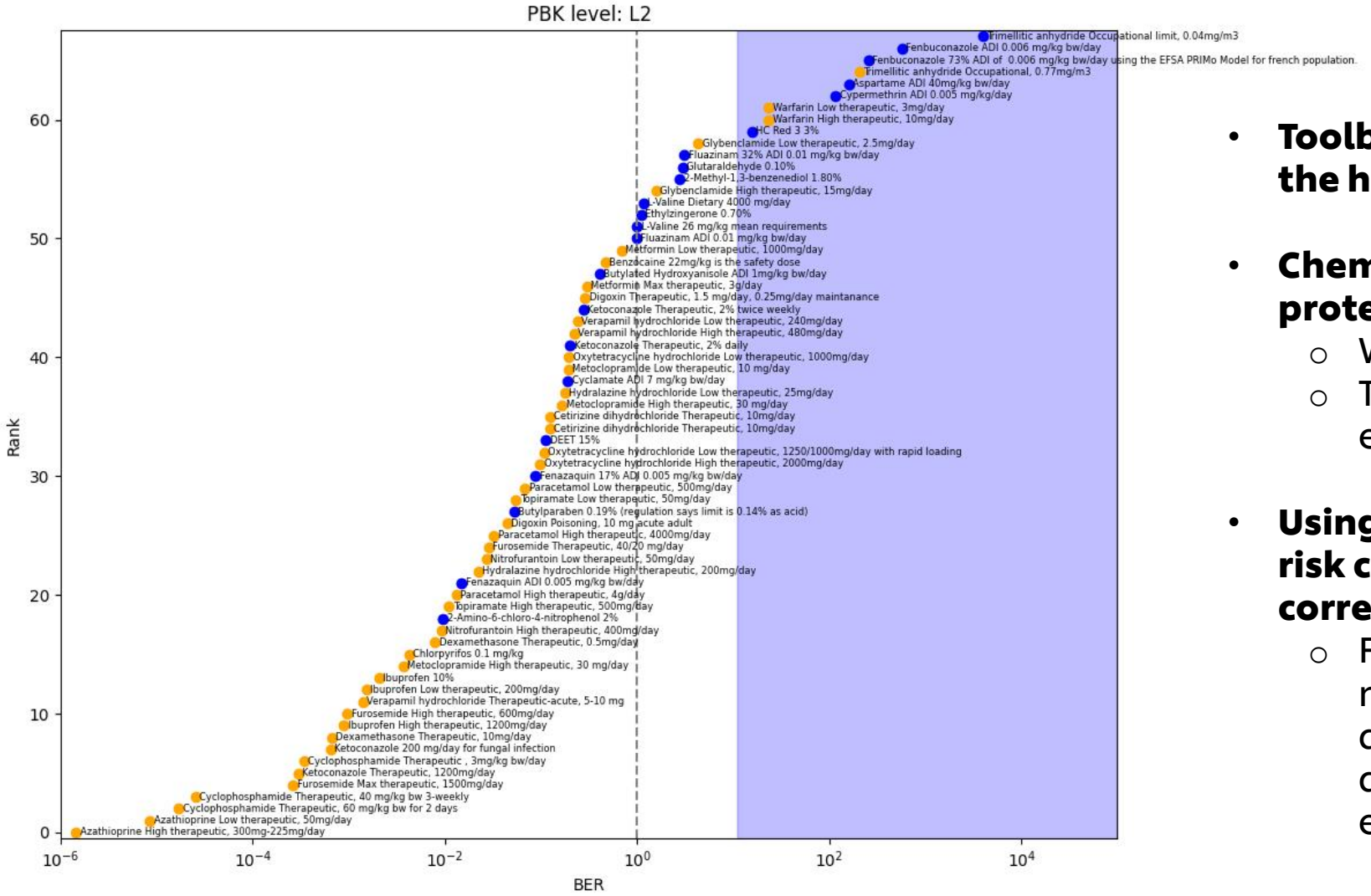
- Oral, dermal, IV and inhalation exposure scenarios

- Organ toxicities, CNS disruptions, immune system dysregulation, non-specific effects, blood-based disorders etc...

Unilever

NAM Systemic toolbox remains protective (93%) when 38 additional chemicals and 70 exposure scenarios were tested

Extended evaluation (Cable et al., 2025)



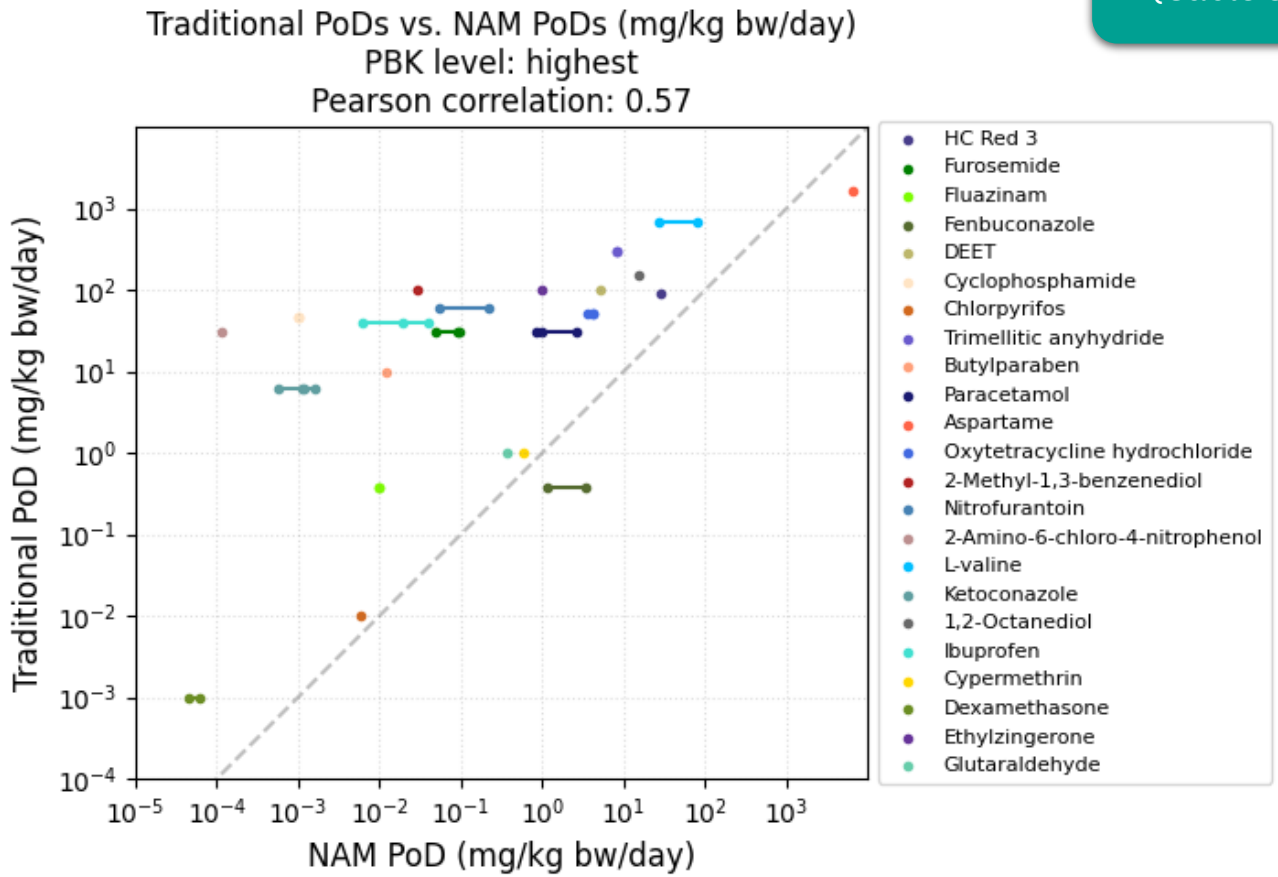
- **Toolbox not protective for 3/46 of the high-risk exposure scenarios**
- **Chemical- Exposure scenarios not protective for:**
  - Warfarin therapeutic oral dose
  - Trimellitic anhydride inhalation exposure
- **Using BER >11, only 27% of the low-risk chemical-scenarios would be correctly identified as such**
  - For the other 73%, refinement is needed (i.e. Approaches to distinguish bioactivity from adversity; refine exposure estimates etc.).

Cable et al., 2025: https://doi.org/10.1093/toxsci/kfae159

NAM PoDs are more conservative (i.e. lower) than the minimum in vivo PoD

Extended evaluation (Cable et al., 2025)

- For 25 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
- Reverse dosimetry was performed to transform PODNAM in µM to an external dose in mg/kg/day
- The range reflects that for some chemicals more than 1 exposure scenario was assigned



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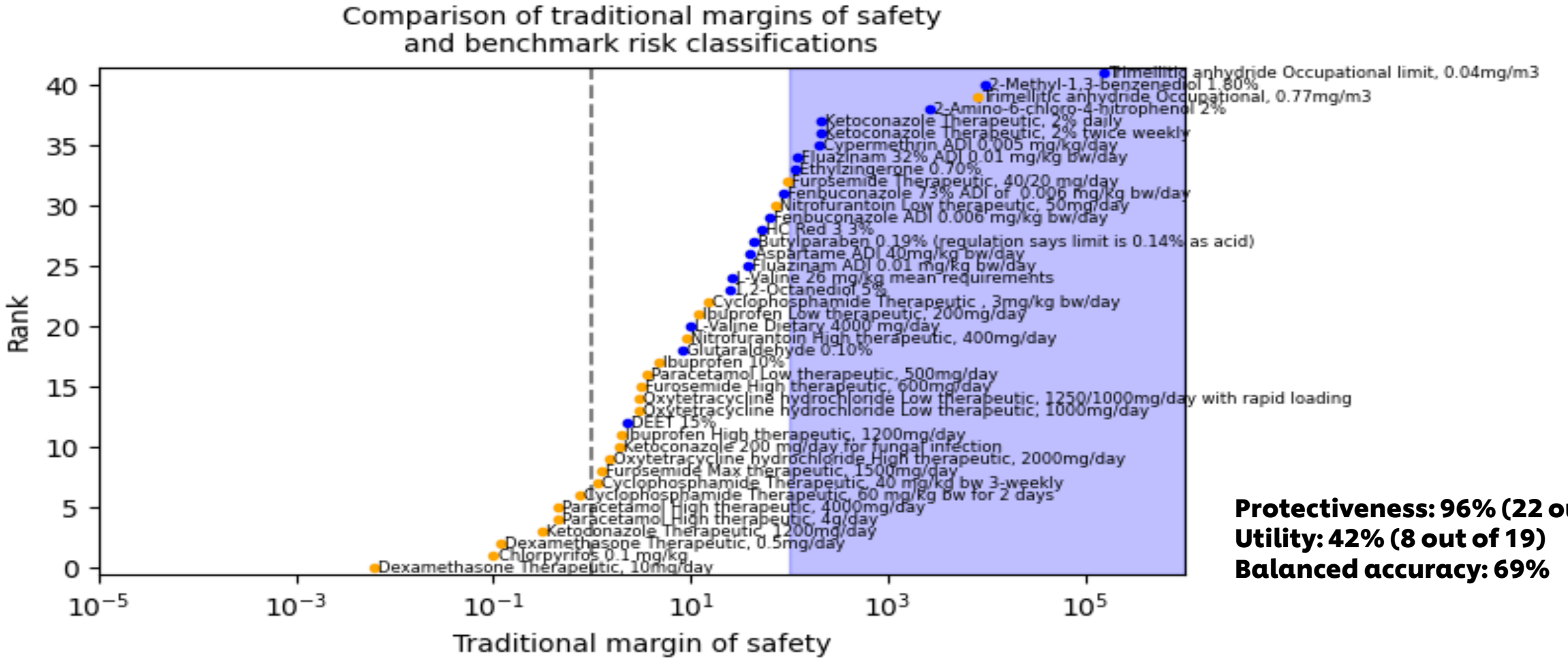
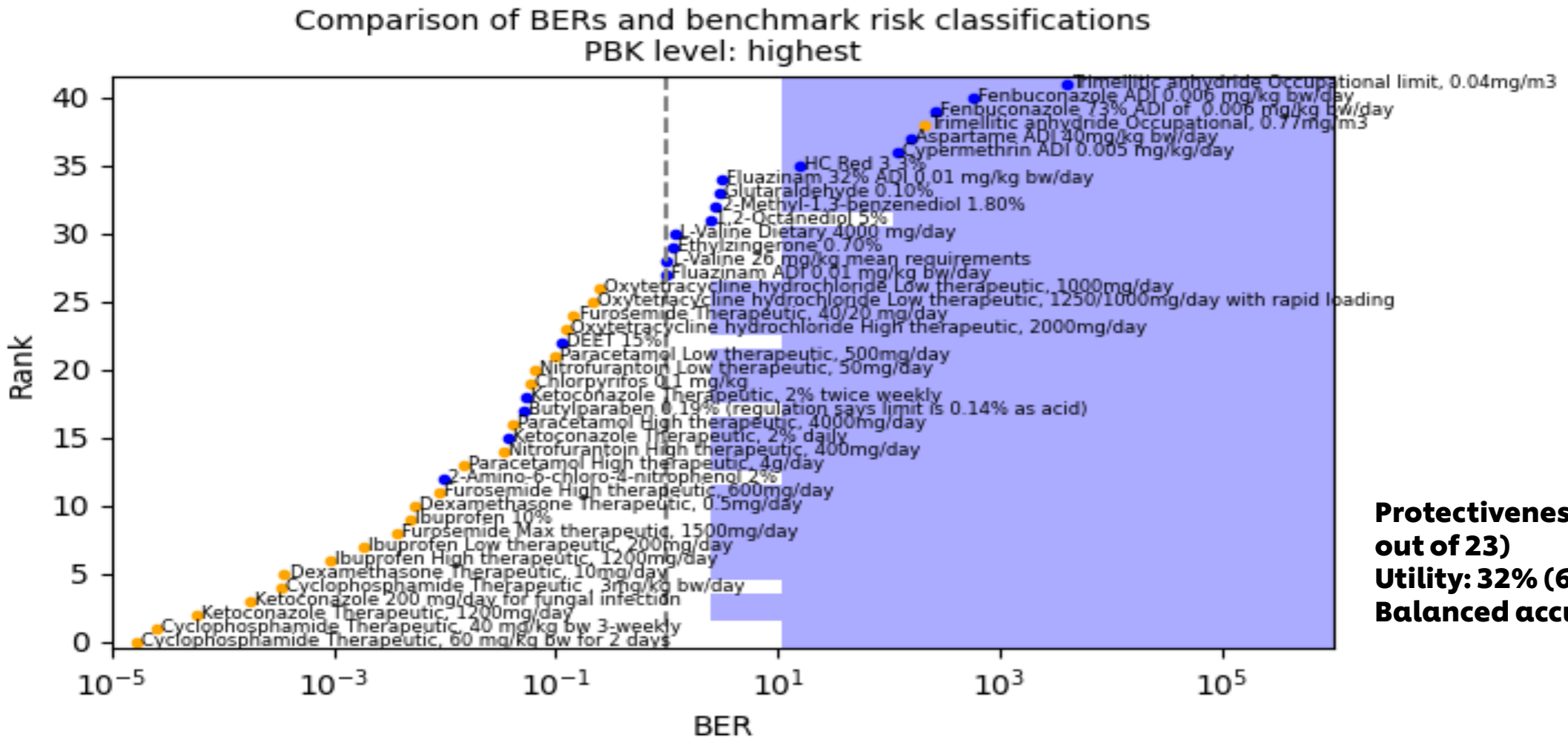
The protectiveness and utility of the traditional approach was calculated to be 97% and 42% when using the lowest in vivo NOEL/NOAEL and a Margin of Safety of 100



Protectiveness: 96% (22 out of 23)
Utility: 42% (8 out of 19)
Balanced accuracy: 69%

Cable et al., 2025: https://doi.org/10.1093/toxsci/kfae159

For the same chemicals, the performance of the NAM-based toolbox was equivalent (96% protectiveness and 32% utility)



Protectiveness: 96% (22 out of 23)
Utility: 32% (6 out of 19)
Balanced accuracy: 64%

Cable et al., 2025: https://doi.org/10.1093/toxsci/kfae159

Key findings from the evaluations

- The toolbox is protective for a wide range of chemicals and could be used within a weight of evidence risk assessment framework.
- PODNAM are conservative for most of the chemicals.
- For majority of the chemicals, the lowest PoD was obtained from the transcriptomics when using the gene-level PoD, followed by IPP.
- Systemic Toolbox is protective for high-risk chemicals despite not always capturing the MoA.
- For chemicals with a specific MoA, IPP is able to detect if the target is present in the panel.
- Generic PBK models might be insufficient to provide more accurate predictions for chemicals which are substrates of transporters.
- Dose-response modelling for transcriptomics leads to a high number of false positives – i.e. low risk exposure scenario being classified as uncertain risk.

Application of NGRA to the evaluation of Climbazole as a cosmetic ingredient

Unilever

Climbazole: Objectives and Approach

- Climbazole is an active ingredient used in several consumer products. We know that bioactivity-based NGRA can result in very conservative safety decisions, so the objective of this case study was to:
- Assess whether a tiered NGRA approach is sufficiently protective *and also useful* to assess the safety of a regulated cosmetic ingredient

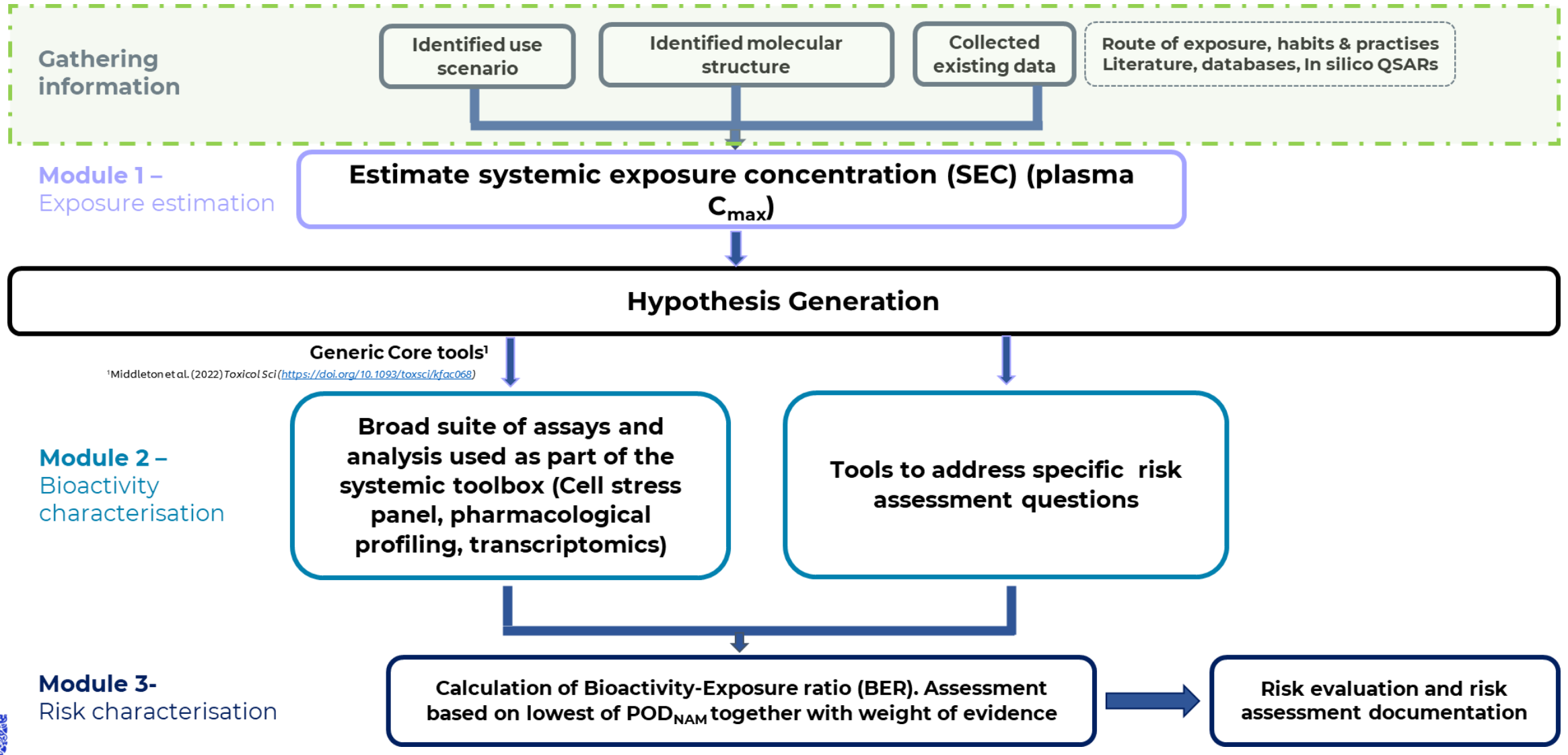
Is Climbazole safe when used at 0.2% in a face cream?

Unilever

Climbazole: Rules and Assumptions

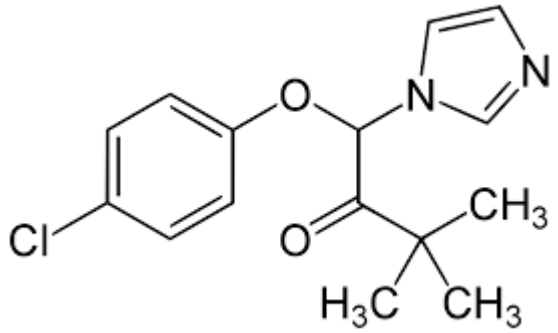
- **For the purposes of this exercise, it has been assumed that no *in vivo* animal data exist on the ingredient**
- **Focus on systemic toxicity**
- **Stand-alone illustration of how to assess systemic toxicity effects (not including genetic toxicity) using NAMs**

Climbazole: Overall approach



Climbazole: Use Scenario and Molecular Structure

- Climbazole (CAS 38083-17-9) has been used in Europe in cosmetics for decades as an anti-dandruff agent or preservative. It is currently regulated under Annex V of the Cosmetic Regulation and approved for use at up to 0.2% as a preservative in leave-on cosmetics.
- It is also approved for at up to 2% in rinse-off shampoo formulations.
- **The specific use scenario** of this case study is for **dermal application of a leave-on face cream formulation** containing **Climbazole at 0.2% w/w**



Daily use of face cream:

- **Amount applied = 1.54 g/day** ***derived from the SCCS Notes of Guidance 2023**
- **Concentration in the finished product = 0.2%**

Unilever

Climbazole: Alerts from *in silico* tools

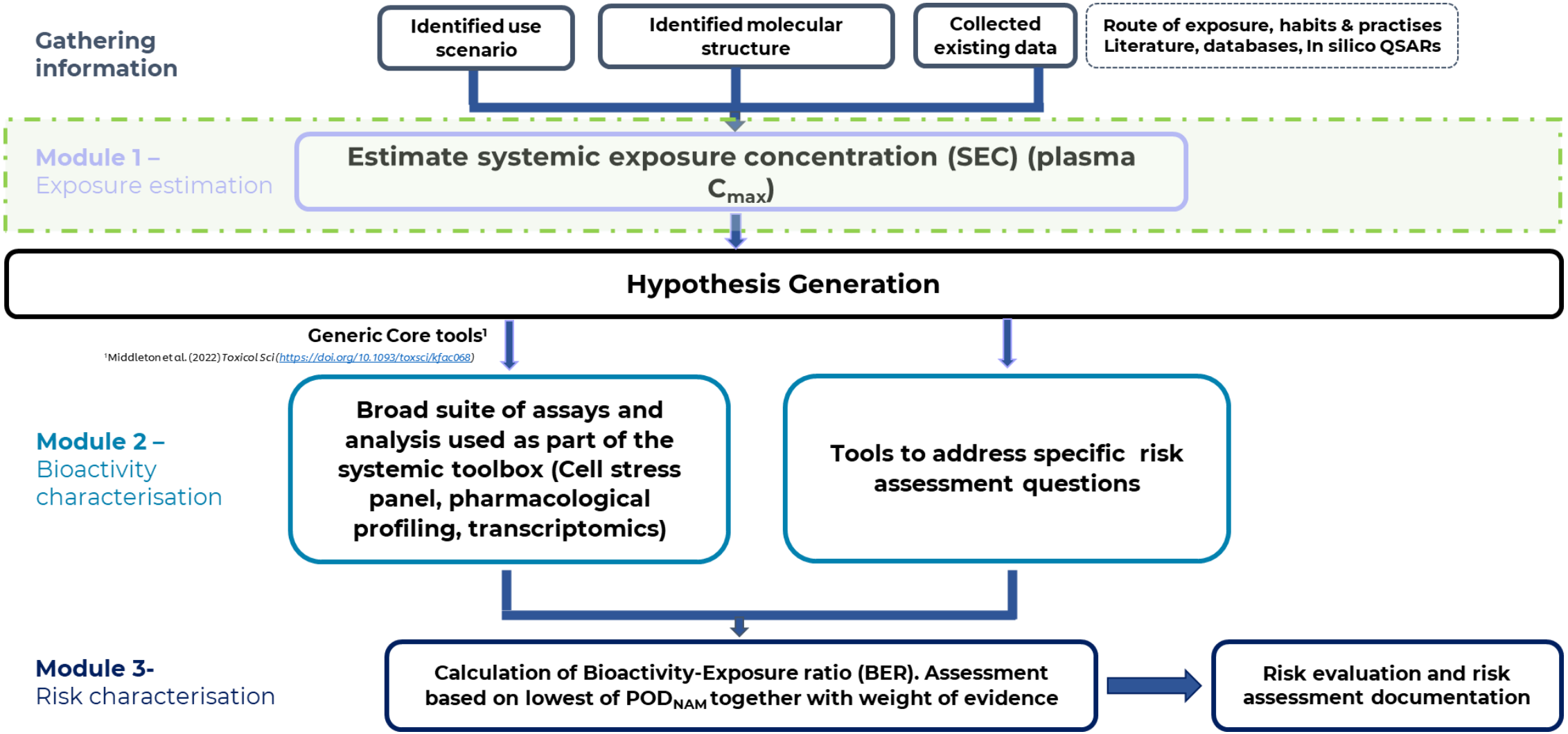
- **DEREK Nexus** — likely toxicity based on chemical structure
- **METEOR Nexus** — possible biotransformation based on chemical structure
- **OECD QSAR Toolbox.** — possible mechanisms of action
- **TIMES** — likelihood of skin sensitisation of the parent and metabolites
- **OPERA** — physchem, environmental fate, range of human-relevant toxicity endpoints
- **VEGA** — physchem, human-relevant toxicity endpoints

AFSA training on predictive chemistry: https://youtu.be/rLWaSgGFGCI

Climbazole: Alerts from *in silico* tools

- Climbazole was within the domain of all models used.
- Climbazole was predicted to have a high order of toxicity, Cramer Class III
- Alerts for hepatotoxicity and protein binding were flagged across DEREK Nexus and the OECD QSAR Toolbox
- There were no alerts for mutagenicity or genotoxicity, however DEREK Nexus and the ISS model within the OECD QSAR Toolbox flagged alerts for carcinogenicity.
- There were no alerts for binding to either ER or AR.
- Climbazole flagged alerts for adrenal gland toxicity and reproductive and developmental toxicity.

Climbazole: Overall approach



Climbazole: Exposure Estimation

Exposure scenario and target individual/population

Product type:	**Face cream**
Amount used per day (g/day):	1.54
Frequency of use:	2.14 times per day
Ingredient inclusion level:	0.2%
Application site:	½ area head
Skin surface area (cm^2):	565
Target individual	**60 kg European female**
Leave on or rinse off:	Leave on
Amount of ingredient in contact with skin per occasion (mg):	3.08

External applied dose = **0.0513 mg/kg bw/day**
(A x 1000 mg/kg x C/100)/60 = mg/kg bw/day

Unilever

Climbazole: Exposure Estimation

From applied dose to internal concentrations

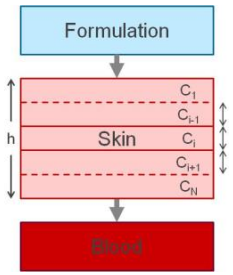
External dose

- Route of exposure
- Consumer use (Habits & Practices)
- Applied dose (external concentration)

ADME parameters

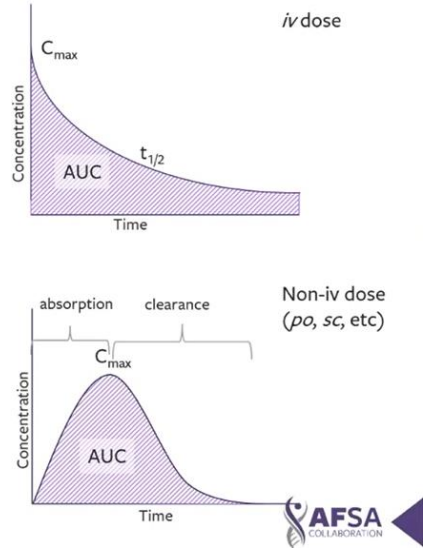
Absorption
Distribution
Metabolism
Elimination

- Skin penetration
- Phys-chem properties
- Hepatic clearance
- Fraction unbound
- Blood:plasma ratio



Kinetic profile of chemical

Physiologically-based kinetic (PBK) modelling – Internal concentration (plasma, urine, organ-level)



Images from: AFSA training module "Dosimetry (Internal Exposure)",2022

https://www.afsacollaboration.org/sciencex_event/dosimetry-internal-exposure-ivive/

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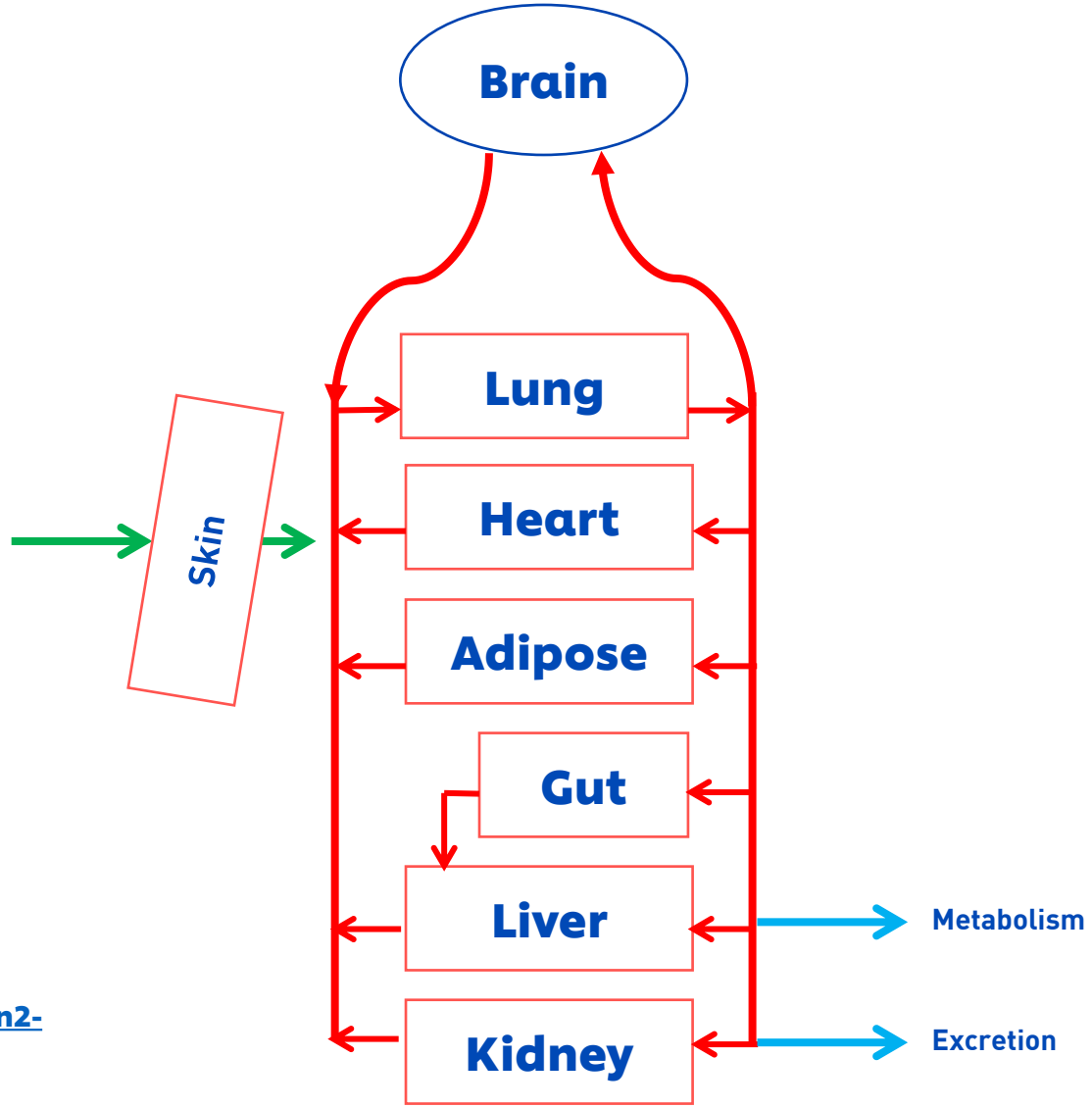
Climbazole: Exposure Estimation

What is PBK modelling?

- **Mathematical description of interconnected compartments representing the human body**
- **Describe ADME (Absorption, Distribution, Metabolism, and Excretion) properties of a chemical within the body**
- **Prediction of concentration in blood, plasma, and tissues over time**
- **Can model an individual or a population**



Links to training materials on PBK modelling:

NURA Dynamic discussions: https://pcrm.widen.net/view/video/xr5ojwu8vo/Session2-DyNAMic-Discussions-2023?x.share=true&x.portal_shortcode_generated=a7lwj1xi&x.app=portals

AFSA: https://youtu.be/UGKEMS6DPRo

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Climbazole: Exposure Estimation

ADME Data Generation

- **In silico tools exists to predict ADME properties from structure (ADMET predictor withing GastroPlus)**
- **The most important ADME properties were generated through in vitro testing:**
  - ***Dermal absorption*: used to derive kinetic parameters for chemical partitioning in the skin layers and absorption through systemic circulation *Low dermal penetration in vitro***
  - ***Blood to plasma ratio*: determines the concentration of the chemical in whole blood compared to plasma and provides an indication of chemical binding to erythrocytes. *Binds RBCs***
  - ***Plasma protein binding*: the degree of binding determines the free available concentration of the chemical in plasma. *High binding to human plasma proteins (97.09%)***
  - ***Metabolic stability*: evaluated using plated primary hepatocytes and it is used to understand the route of elimination of a chemical and derive values for intrinsic hepatic clearance and half-life. *High clearance in the assay***

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Climbazole: Exposure Estimation

ADME Data Generation

	Source
Molecular weight	**292.76 g/mol**
Log P	**ADMET predictor**
pKa	**ADMET predictor**
Fraction unbound in plasma (f_{up})	**Measured**
Blood: plasma ratio	**Measured**
Hepatic intrinsic clearance (L/h)	**Measured**
ECCS classification	***Varma et al., 2015***
Renal excretion	**GFR*Fup**
Dermal absorption parameters: Partition coefficient and diffusivity in skin layers	**Measured, Eurofins,** *Ex vivo* skin penetration study designed according to *Davis et al. 2011* meeting OECD TG 428 and SCCS guidance

Main observations:

- **Very low skin penetration (~1.5% over 24h)**
- **Climbazole was readily cleared in the plateable hepatocyte assay**

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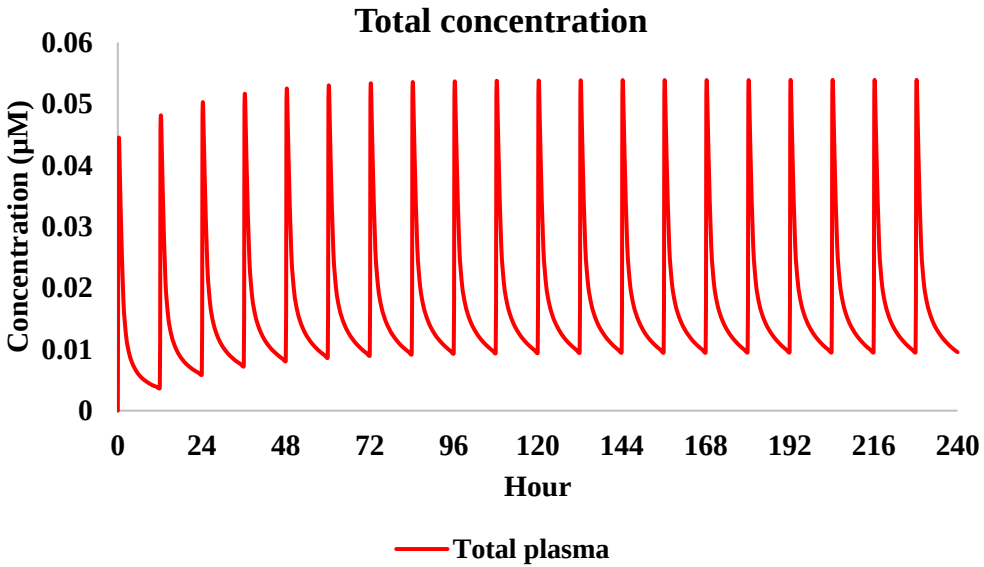
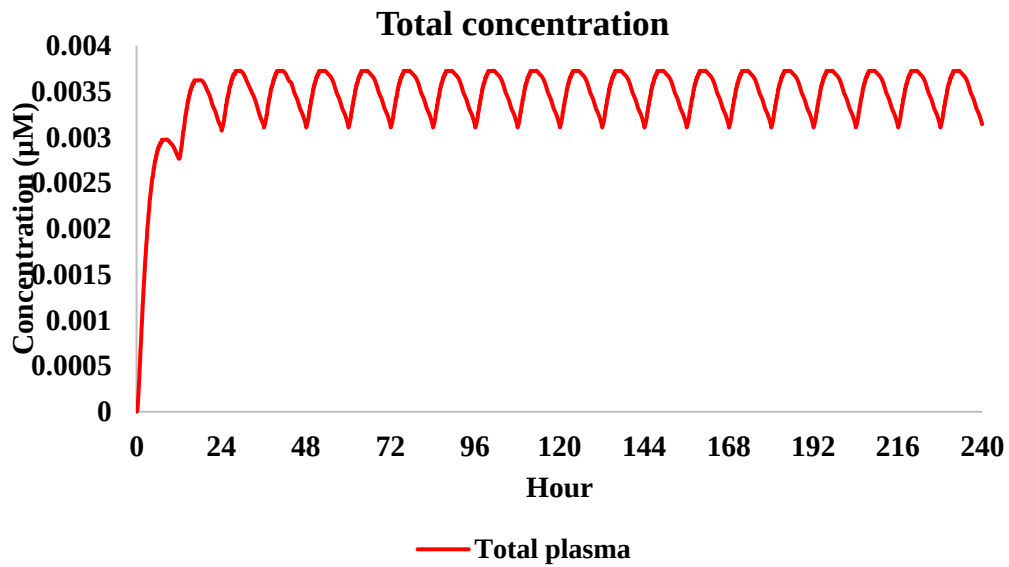
Climbazole: Exposure Estimation

From applied dose to internal concentration

L1 – in silico parameters



L2 – key in vitro parameters



- Overall, upon dermal absorption only a small amount of Climbazole enters systemic circulation, after which the most likely route of elimination is liver clearance of climbazole.
- Following twice daily application of a face cream, climbazole does not appear to accumulate with the PBK model run up to 10 days.
- Refining key ADME parameters using experimental data led to a reduction in the predicted plasma C_{max}

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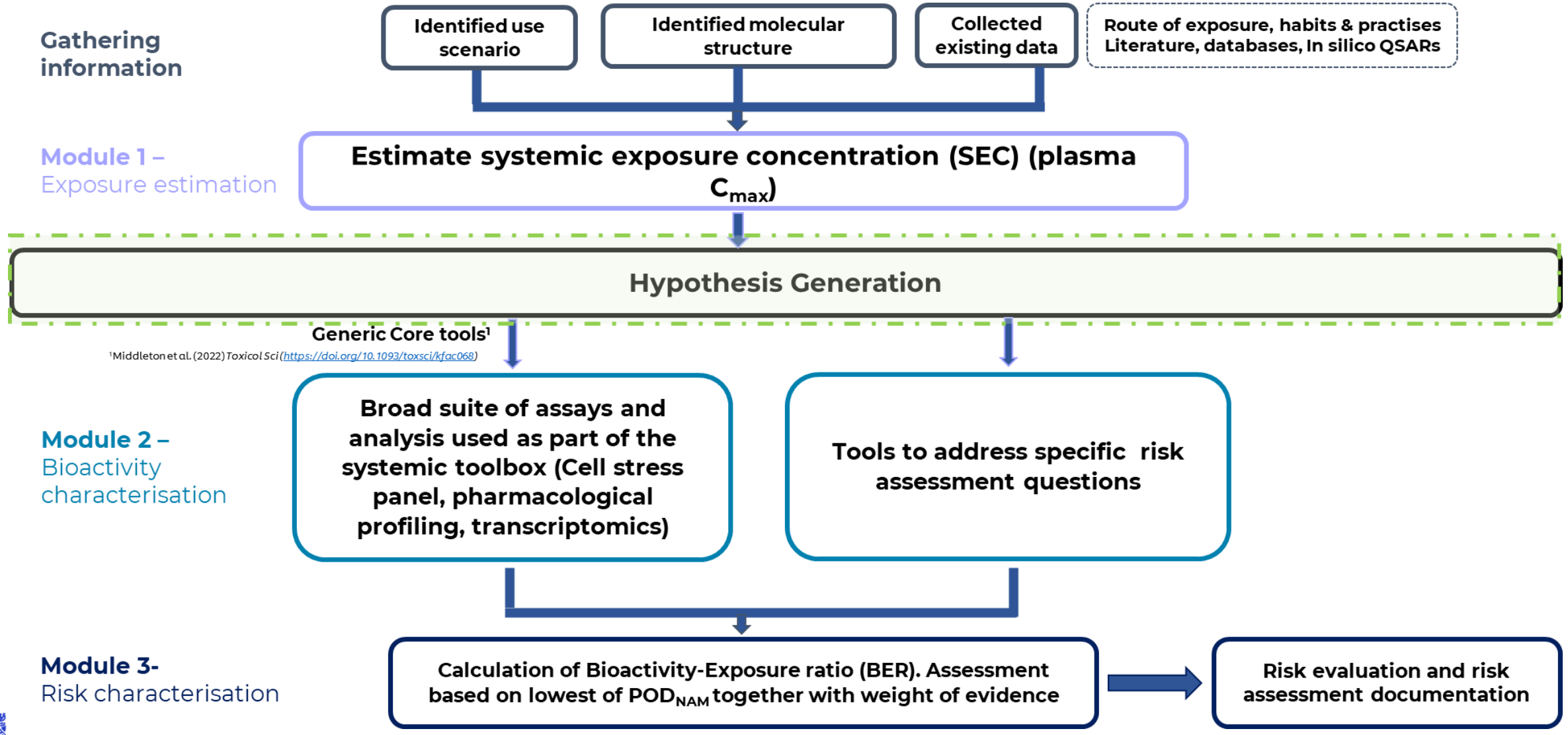
Climbazole: Overall approach



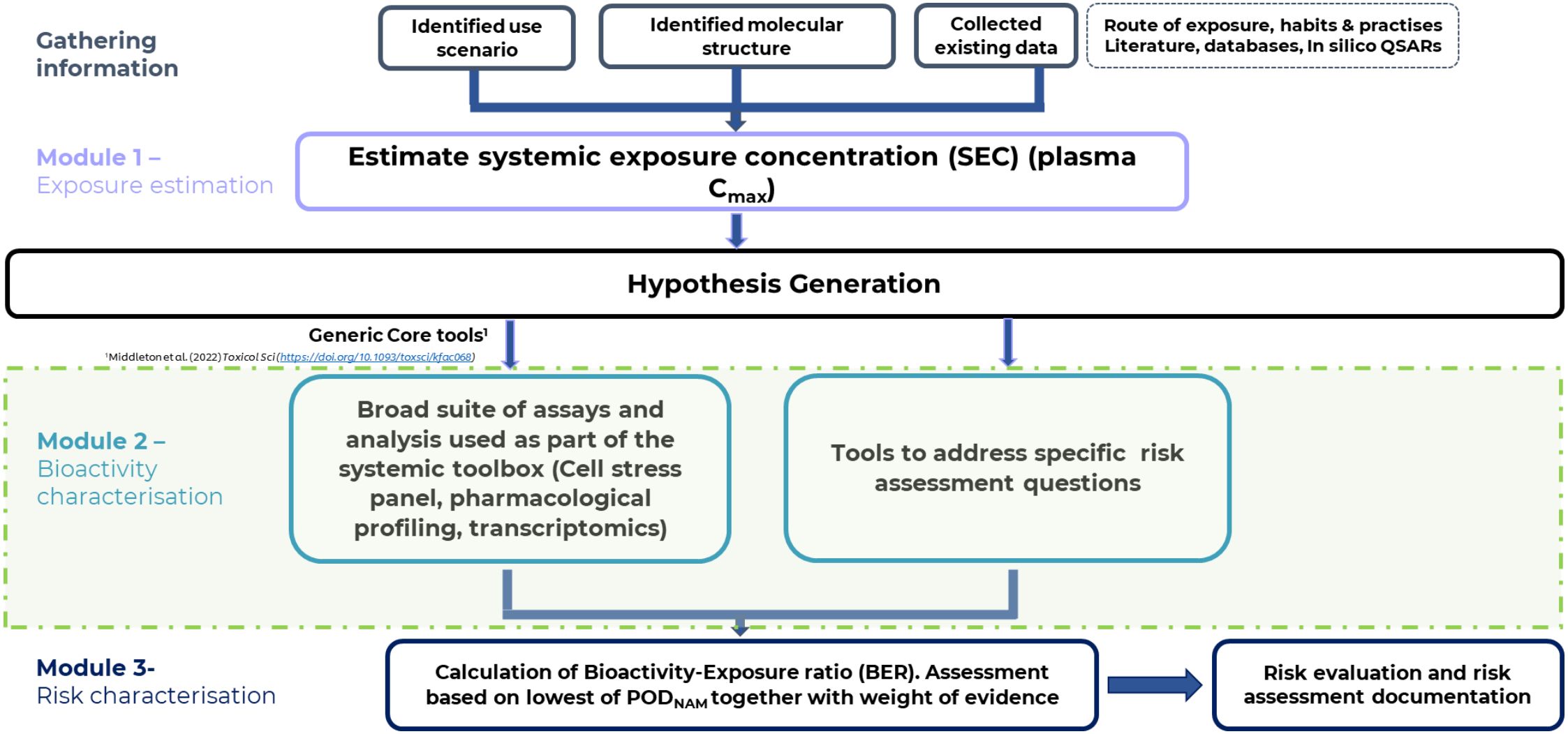
Climbazole: Hypothesis Generation

Hypothesis Generation

- In silico **alerts for hepatoxicity** → covered by cell lines, no specific method so broad screening of activity?

- In silico **alerts for reprotox and adrenal gland toxicity** → Imidazole derivatives are known to inhibit ergosterol synthesis and this is the primary mechanism of action for the anti-fungal efficacy displayed by azole fungicides (other imidazole derivatives, including ketoconazole). Likely to explain the efficacy of climbazole as a preservative and therefore needs investigating.

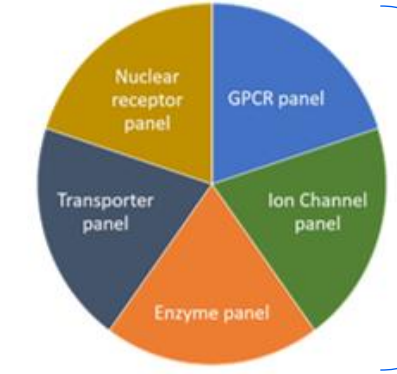
Climbazole: Overall approach



Climbazole: Broad suite of bioactivity assays

Transcriptomics was applied as a broad nontargeted biological screen

In vitro pharmacological profiling



~79 targets


PERSPECTIVES

Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

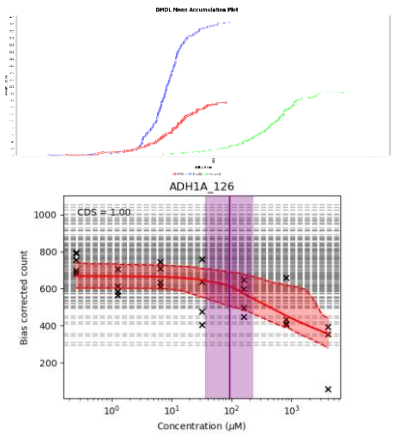
Bowes et al. 2012. Nat Rev Drug Discov 11(12): 909-22

To investigate specific biological activity with 44 key targets involved in drug attrition (Pharma) and additional targets relevant to exposure to cosmetics– now expanded to 79 targets

To characterize non-specific biological activity which is not mediated via a specific protein/receptor interaction

High-Throughput transcriptomics (HTTr)

- TempO-seq technology: full gene panel
- 24hr exposure
- 7 concentrations
- Various cell models (e.g. HepG2, MCF7, HepaRG)
- Dose-response analysis using BMDExpress2 and BIFROST model



Reynolds et al. 2020. Comp Tox 16: 100138
Baltazar et al. 2020. Toxicol Sci 176(1): 236–252

Cell stress panel (CSP)

- 36 biomarkers covering 10 cell stress pathways
- HepG2
- 24hr exposure
- 8 concentrations
- Dose-response analysis using BIFROST model

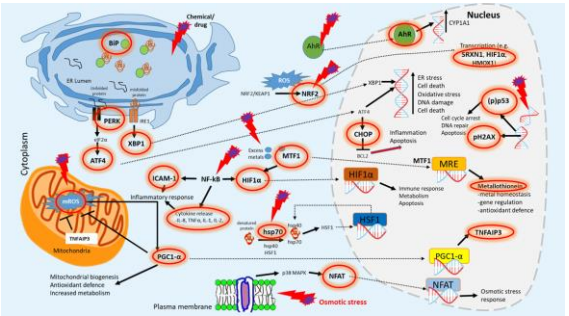


Image kindly provided by Paul Walker (Cyprotex)

Hatherell et al. 2020. Toxicol Sci 176(1): 11-33

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Climbazole: Results from key NAMs

Deriving Points of Departure (PoDs)

HTTr (HepG2, HepaRG, MCF-7)

- Effects on the transcriptome were noticed in all 3 cell lines tested.
- PoDs were calculated using changes at the gene level and changes at a pathway level
- MCF-7 cells were the most sensitive cell line with a gene level PoD of 0.094 µM and a pathway level PoD of 12.9 µM

Cell Stress Panel

- Climbazole was active across all but 3 measured biomarkers.
- PoDs for stress biomarkers correlated with PoDs for cell health biomarkers measured in the same assay and were all around the highest tested dose. Indicating that all effects measured are potentially indicative of the start of cytotoxocity.
- Global PoD calculated to be 12 µM

In vitro Pharmacological profiling

- Screening performed at 10 µM
- ~79 targets compiled by Cosmetics Europe Safety pharmacology WG
- 3 hits: Aromatase, PXR and SLC6A3
    - Dopamine transporter, SLC6A3 **IC50 calculated to be 0.073 µM**
    - **Aromatase IC50 calculated to be 0.091 µM**
    - Pregnance X Receptor IC50 calculated to be 4.9 µM

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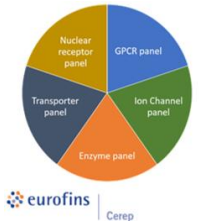
Climbazole: Tools to address specific questions

Tools to address specific risk assessment questions

Derek nexus

VEGA

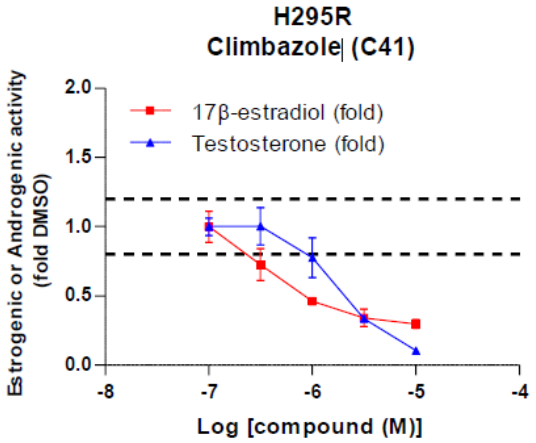
Structural alerts for reprotoxicity and structural similarity to compound known to interfere with sterol biosynthesis



Aromatase inhibition known to affect natural biosynthesis of hormones
Climbazole IC50 : 0.091
Prochloraz IC50: 0.021

Is Climbazole likely to interfere with hormone synthesis in vivo; and if so at what concentration?

Generation of data in OECD 456, H295R steroidogenesis assay coupled to ERa and AR-CALUX



Climbazole demonstrated preferential interference with estradiol production, fits with initial alerts.

Climbazole LOEC = 0.3 µM (ERα CALUX)

Prochloraz LOEC = 0.0001 – 0.1 µM
(Hecker et al., 2018)

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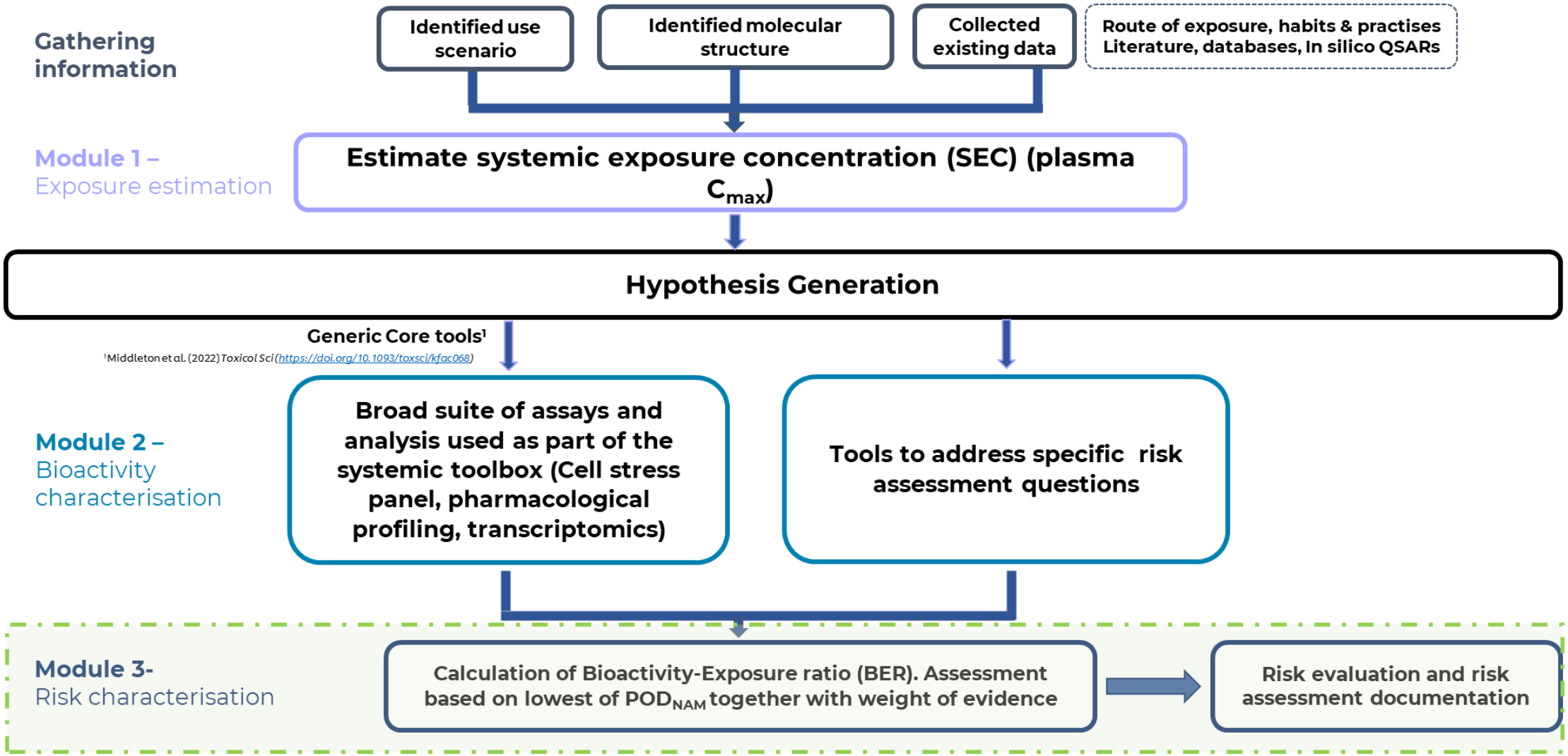
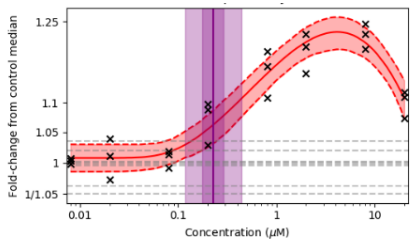
Climbazole: Overall approach



Climbazole: Calculation of the Bioactivity Exposure Ratio (BER)

Point of departure (POD) derived from concentration-response data



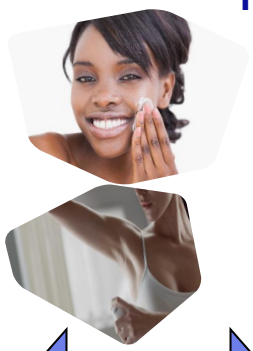
Systemic toolbox of assays (NAMs) which cover a broad biological space – measurements of bioactivity

- **Cellular stress assays**
- **Transcriptomics**
- **Receptor binding/enzymatic assays**
- **Others**

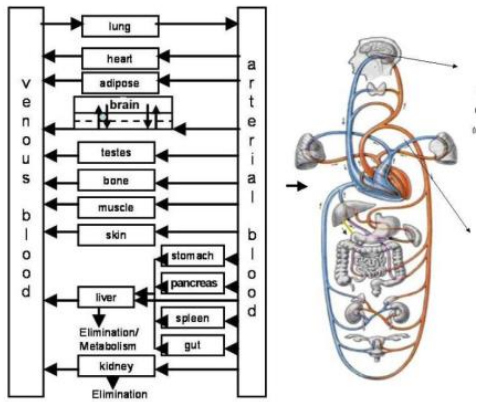
Calculation of Bioactivity exposure ratio (BER)

The BER is defined as the ratio between the POD and the relevant exposure metric

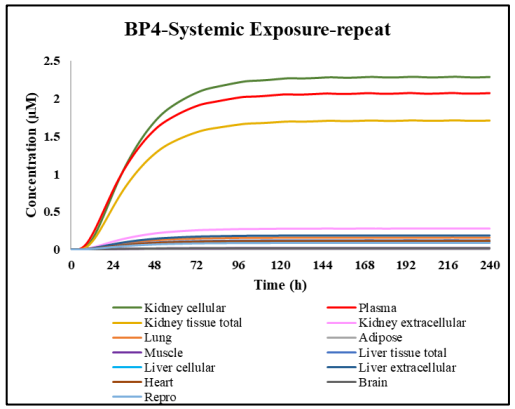
Exposure models (PBK, free/total concentration)



Skin pen



Exposure estimation: Plasma C_{max}, organ distribution, AUC



Climbazole: Calculation of the Bioactivity Exposure Ratio (BER)

NAM	PoD (µM)	BER (using L1 C_{max})	BER (using L2 $_{Cmax}$)
In Vitro Pharmacological Profiling	0.073	1.4	19.7
Cell Stress Panel	12	222.2	3243.2
BIFROST HTTr MCF-7	0.094	1.7	25.4
BIFROST HTTr HepG2	0.72	13.3	194.6
BIFROST HTTr HepaRG	0.34	6.3	91.9
BMD Pathway HTTr MCF-7	12.9	238.9	3486.5
BMD Pathway HTTr HepG2	48.4	896.3	13081.1
BMD Pathway HTTr HepaRG	48.1	890.7	13000.0
H295R ER-CALUX LOEC	0.3	5.6	81.1
H295R AR-CALUX LOEC	1.0	18.5	270.3

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Climbazole: Risk assessment conclusion

Qualitative assessment of uncertainties

Area	Level of certainty (rationale)	Is value likely to be an over- or under-estimate (rationale)	Impact on risk assessment decision

Areas

- **Consumer exposure (applied dose)**
- **Identification of metabolites**
- **Consumer exposure (Internal dose)**
- **Range of biomarkers assessed**
- **Use of short-term tests *in vitro* to inform about risks of long-term human exposure**
- **Point of departure selection**

Similar approach to OECD (2021): IATA for Phenoxyethanol

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Climbazole: Risk assessment conclusion

Qualitative assessment of uncertainties- an example

Area	Level of certainty (rationale)	Is value likely to be an over- or under-estimate (rationale)	Impact on risk assessment decision
Range of biomarkers assessed	**Moderate** There is increasing evidence that POD_{NAM} obtained from the core NAMs, IPP, CSP and HTTr are protective for a range of chemicals (Middleton *et al.,* 2022) and previous case studies (Baltazar *et al.,* 2020, OECD phenoxyethanol). The hypothesis and exposure driven approach led to the inclusion of an additional NAM to investigate the steroidogenic activity and benchmark the potency of the response.	**Climbazole showed potential for specific activity through the structural alerts flagged at the in silico stage and the specificity of some of the bioactivity results. This was covered in the NAMs used and a PoD derived. Broad spectrum NAMs showed overall high activity for climbazole in the test systems with leading PoDs derived from gene level changes in the HTTr, which is likely conservative given the low number of genes changing at low concentrations.**	There are remaining uncertainties regarding the protectiveness of the tools utilised for a broader range of chemistries. **Confidence could be increased by assessing how protective the range of biomarkers are for many more compounds** and whether different biomarkers are needed to ensure the *in vitro* PoD is protective compared with the *in vivo* PoD.

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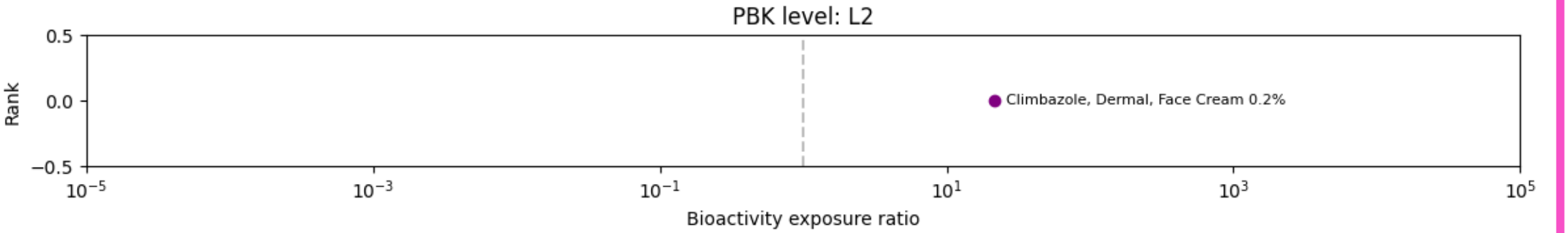
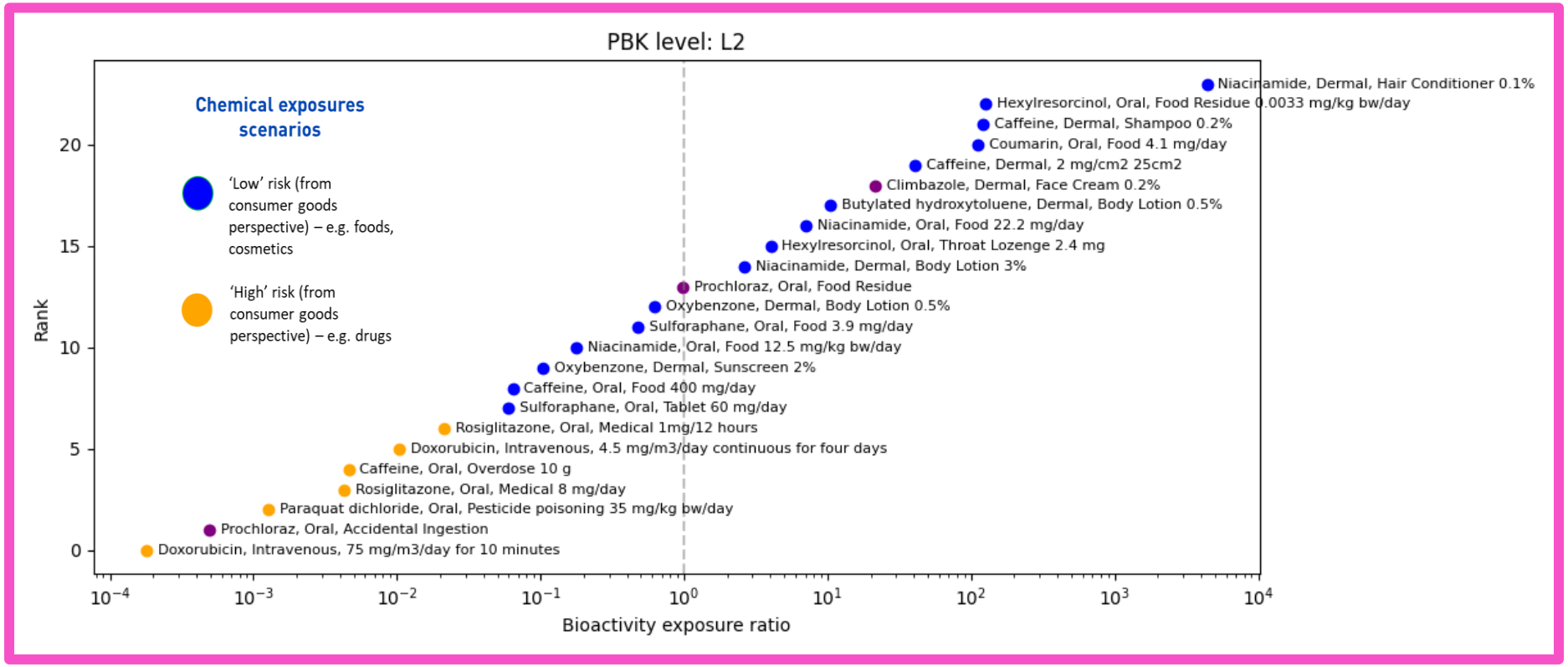
Climbazole: Risk assessment conclusion
Interpreting the BER using the lowest PoD_{NAM} and the deterministic BER



Climbazole: Risk assessment conclusion
Interpreting the BER using the lowest PoD_{NAM} and the deterministic BER

What if the same approach was applied to other chemicals with varying risk classifications?



Note: Low risk is different than low toxicity; it is all about integrating exposure.

Conclusions & reflections

NAM-based assessment for 0.2% inclusion of Climbazole

Lowest BER= 19.7

BER range= 19.7-13000

Conclusion

Low risk considering weight of evidence and model/PoD relevance

Traditional animal assessment for 0.2% inclusion of Climbazole

NOAEL= 5 mg/kg bw/day

Exposure= 0.0138 mg/kg bw/d

Margin of Safety (MoS)= 362

Conclusion

Low risk – MoS > 100

(SCCS opinion)

NAM-based risk assessments are in generally more conservative than traditional approaches

- Middleton et al. (2022) *Toxicol Sci* (*https://doi.org/10.1093/toxsci/kfac068*)
- Reardon A et al., 2023 https://doi.org/10.3389/ftox.2023.1194895
- Zobl et al., 2023 http://dx.doi.org/10.14573/altex.2309081
- Paul-Friedman K et al., 2020: https://doi.org/10.1093%2Ftoxsci%2Fkfz201
- Baltazar MT et al., 2020: http://dx.doi.org/10.1093/toxsci/kfaa048
- Ebmeyer et al., 2024: https://doi.org/10.3389/fphar.2024.1345992
- Cable et al., 2025: https://doi.org/10.1093/toxsci/kfae159

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Conclusions and Reflections

- Showcased a range of in silico and in vitro NAMs that can be used for safety decision making for systemic toxicity
- The method is exposure-led and follows a tiered approach for both exposure and bioactivity
- Bespoke NAMs can be added to the NGRA to fill gaps identified along the process
- 'Early tier' in vitro screening tools show promise for use in a protective rather than predictive capacity.
- NGRA requires a mindset shift and a multidisciplinary team

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