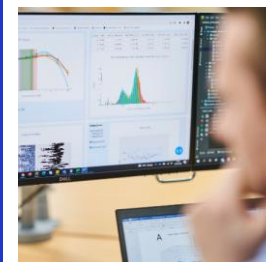# Introduction on Crucial Role of Exposure Assessment in NGRA for Humans and Environment

Predrag Kukic, Unilever SERS, UK

SERS
Safety, Environmental
& Regulatory Science
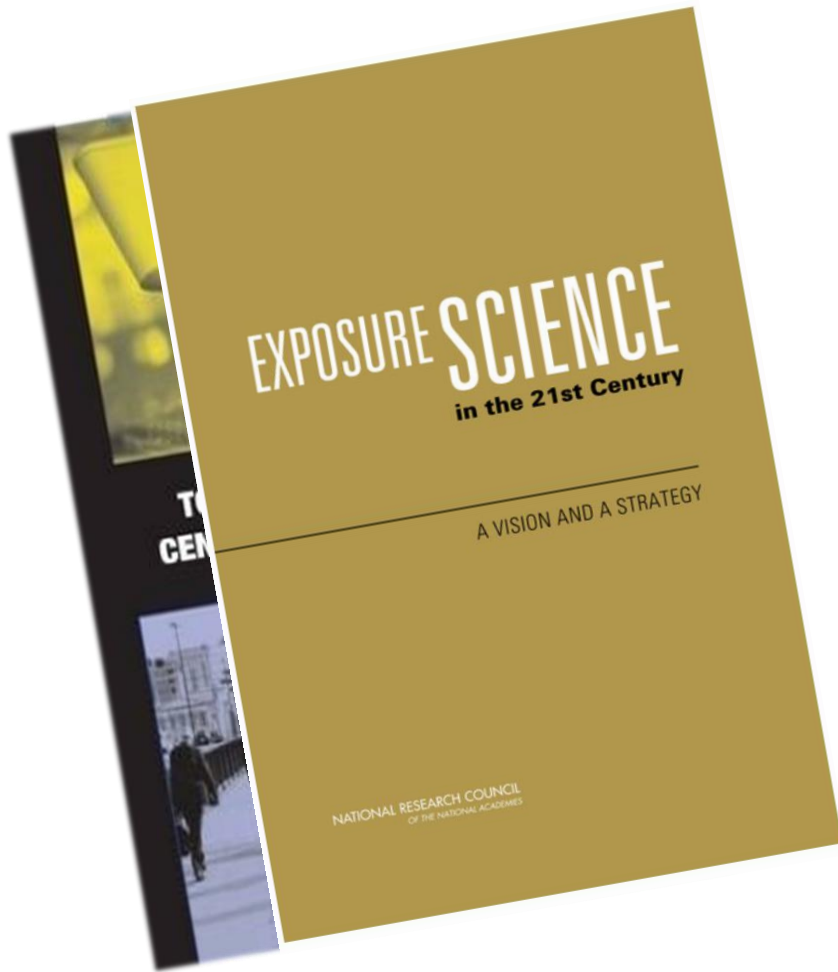
Why do we generate (eco)toxicology data?

Or

If our goal is to protect **people** and the **environment**, exposure data are critical

Images generated by CoPilot

The Paradigm Shift

Distributions of Oral Equivalent Values and Predicted Chronic Exposures

Do we need to be able to predict adverse outcomes or is it more useful and relevant to know that under specified exposure conditions, an adverse outcome is not likely to happen?

Protection Not Prediction

2007 Toxicity Testing in the 21st Century (TT21C)

"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 biological processes using cells, cell lines, or cellular components, preferably of human origin."

Perturbation of 'toxicity pathways' and stress responses

What is Next Generation Risk Assessment (NGRA)?

"An exposure-led, hypothesis driven risk assessment approach that incorporates one or more NAMs to ensure that chemical exposures do not cause harm"

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

First workflow for *ab initio* NGRA

TIER 0: *IDENTIFY USE SCENARIO, CHEMICAL OF CONCERN AND COLLECT EXISTING INFORMATION*

1. IDENTIFY USE SCENARIO
2. IDENTIFY MOLECULAR STRUCTURE → EXIT TTC
3. COLLECT EXISTING DATA
4. IDENTIFY ANALOGUES, SUITABILITY ASSESSMENT AND EXITING DATA → EXIT READ-ACROSS

TIER 1: *HYPOTHESIS FORMULATION FOR AB INITIO APPROACH*

5. SYSTEMIC BIOAVAILABILITY (PARENT VS. METABOLITE(S), TARGET ORGANS, INTERNAL CONCENTRATION) → EXIT INTERNAL TTC
6. MOA HYPOTHESIS GENERATION (WEIGHT OF EVIDENCE BASED ON AVAILABLE TOOLS)

TIER 2: *APPLICATION OF AB INITIO APPROACH*

7A. TARGETED TESTING
7B. BIOKINETIC REFINEMENT (IN VIVO CLEARANCE, POPULATION, IN VITRO STABILITY, PARTITION)
8. POINTS OF DEPARTURE, IN VITRO IN VIVO EXTRAPOLATION, UNCERTAINTY ESTIMATION, MARGIN OF SAFETY → EXIT AB INITIO
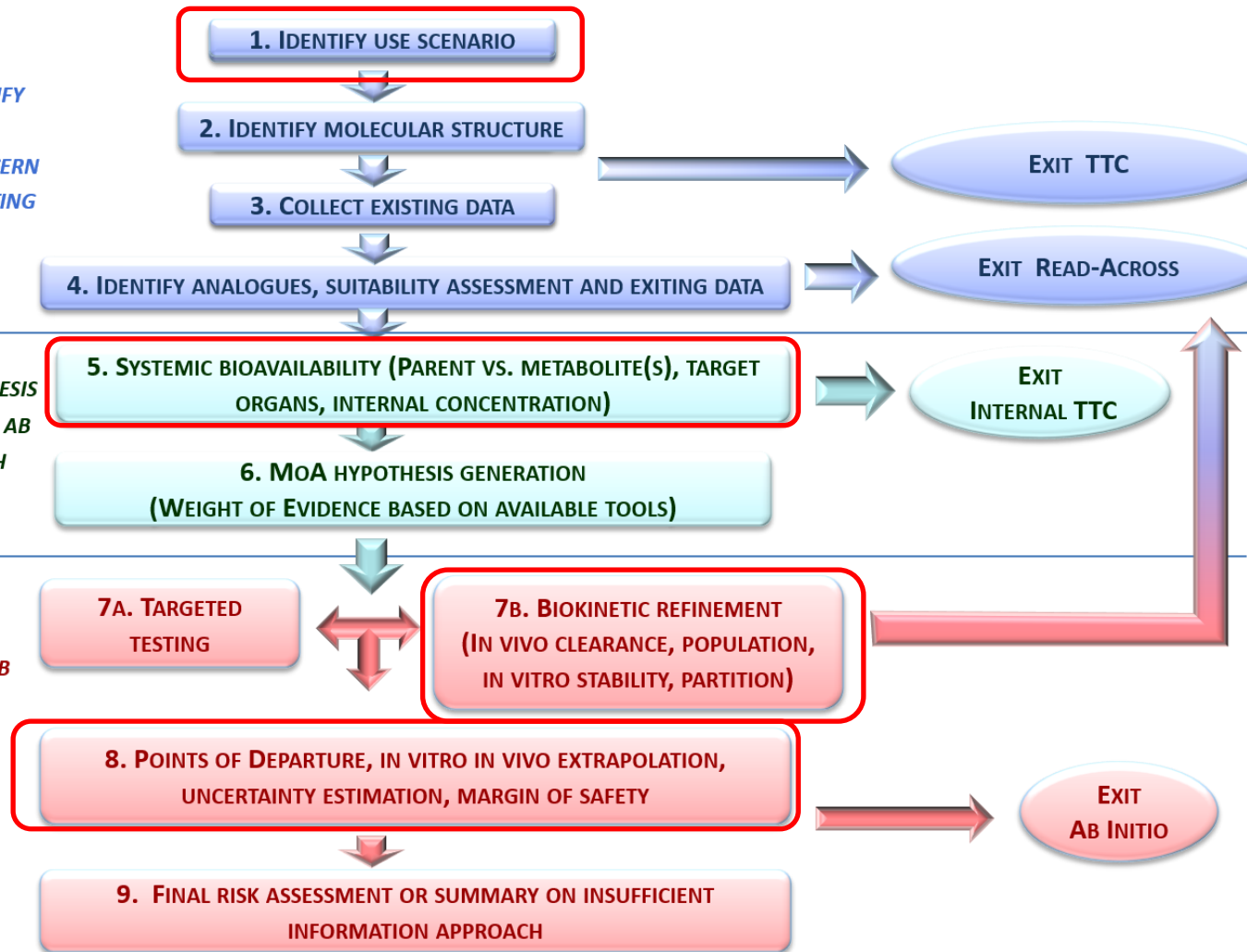9. FINAL RISK ASSESSMENT OR SUMMARY ON INSUFFICIENT INFORMATION APPROACH

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

Some bioactivity NAMs

High-throughput Transcriptomics (HTTr)

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

BMDexpress 2

~70% gene level overlap

Rajagopal et al., 2022
Janowska-Sejda et al.2022

devTOX quickPredict™

- human iPSC cells
- metabolic perturbation of the biomarker's ornithine and cystine
- predicts concentration at which a test article shows developmental toxicity potential (dTP).

Stemina
BIOMARKER DISCOVERY

Toxicology in vitro (2020) Apr 1;174(2):189-209

ReproTracker®

- human iPSC cells
- differentiated into cardio-myocytes, hepatocytes and neuronal rosettes
- Dose depended changes of lineage-specific gene biomarkers are measured to identify potentially teratogenic effects.

toxys

Birth defects Res. (2022) Nov 15;114(19):1210-1228.

Toolbox: cell stress, IPP, HTTr

PBK

pregnant | non-pregnant

- gametogenesis
- fertilisation
- implantation
- embryonic development
- postnatal and multigeneration

Cell Stress Panel (CSP)

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

cyprotex

Cell stress is a fundamental factor in many adverse outcome pathways (AOPs) relating to DART and has been reported as a key characteristic of male and female reproductive toxicants (Azuarga et al., 2019; Luderer et al., 2019)

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

H295R steroidogenesis assay

- human adenocarcinoma cell line NCI-H295R and U2-OS
- *in vitro* effect-based responses of compounds using the H295R steroidogenesis assay coupled to two CALUX® bioassays as a read-out: the ERα and AR CALUX®
- OECD Test No. 456

BDS

Toxicological Sciences, 2023, 194(2), 191–208

In vitro Pharmacological Profiling (IPP)

PERSPECTIVES

Nuclear receptor panel | GPCR panel

51 out of the 75 targets are DART relevant. This includes 20 nuclear hormone receptors, 6 DNT targets, aromatase …

eurofins | Cerep

From principles to application

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

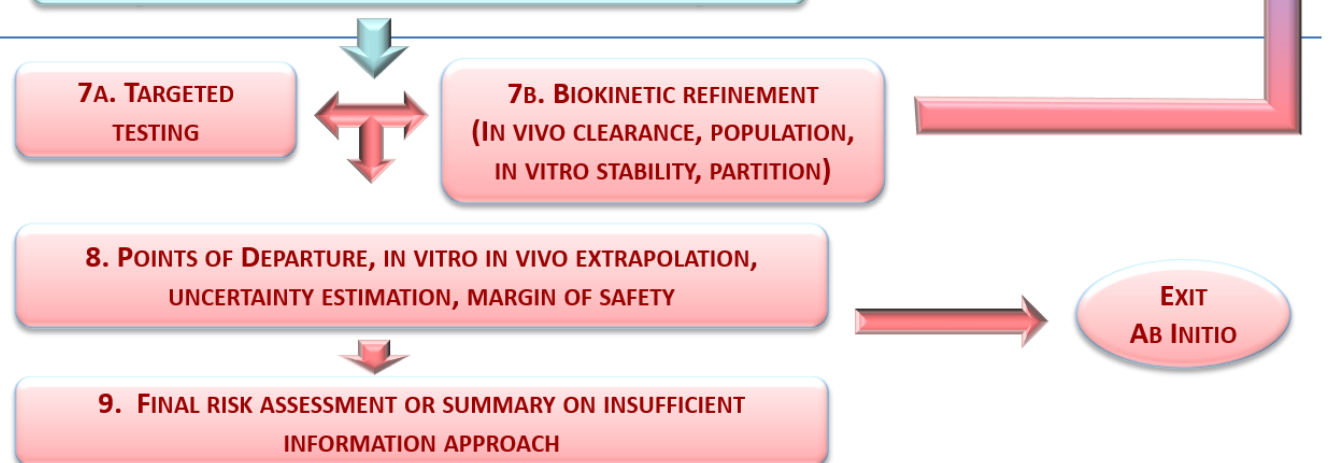**TIER 0:** IDENTIFY USE SCENARIO, CHEMICAL OF CONCERN AND COLLECT EXISTING INFORMATION

1. IDENTIFY USE SCENARIO

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EXIT TTC

4. IDENTIFY ANALOGUES, SUITABILITY ASSESSMENT AND EXITING DATA

EXIT READ-ACROSS

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EXIT AB INITIO

9. FINAL RISK ASSESSMENT OR SUMMARY ON INSUFFICIENT INFORMATION APPROACH

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 embryo assays

Pathway modelling

Human studies

Note - not universally considered a non-animal approach

8

Risk Assessment Outcome

BIOACTIVITY

In vitro pharmacological profiling

Nuclear receptor panel / GPCR panel / Ion Channel panel / Enzyme panel / Transporter panel

~79 targets

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

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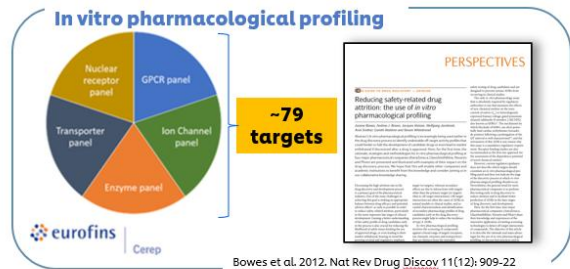
Bowes et al. 2012. Nat Rev Drug Discov 11(12): 909-22

High-Throughput transcriptomics (HTTr)

- TempO-seek technology – full gene panel
- 24hr exposure
- 7 concentrations
- Various cell models (e.g. HepG2, MCF7, HepaRG)
- Dose-response analysis using BMDExpress2 and BIFROST model
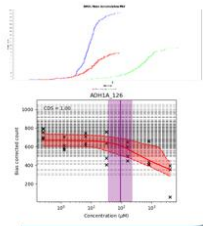
Reynolds et al. 2020. Comp Tox 16: 100138
Baltazar et al. 2020. Toxicol Sci 176(1): 236–252

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)
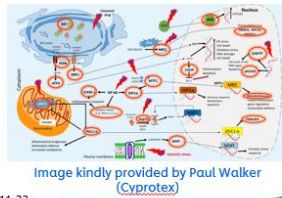
Hatherell et al. 2020. Toxicol Sci 176(1): 11-33
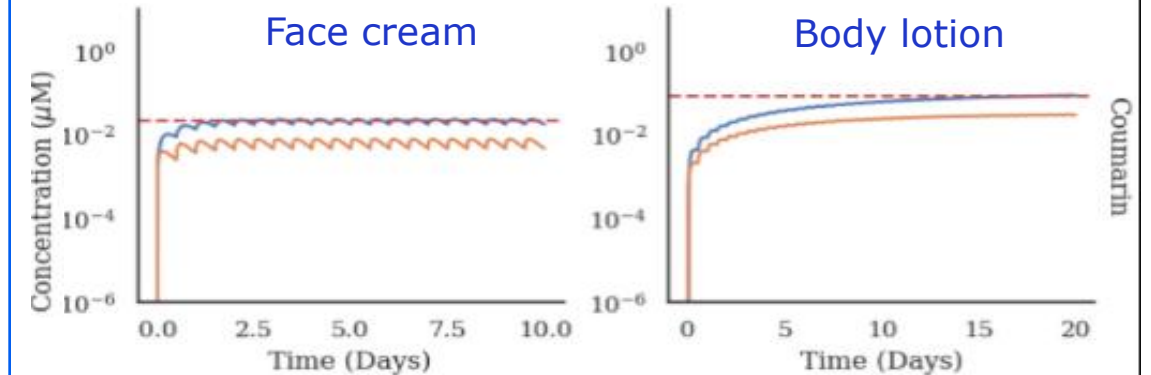
Identify lowest (most sensitive) point of departure, expressed in µM

EXPOSURE

Face cream

Body lotion

Coumarin

(x-axis) Time (Days), (y-axis) Concentration (µM)

Identify realistic worst-case plasma exposure (C_{max}) expressed as µM

BIOACTIVITY EXPOSURE RATIO = $\dfrac{\text{BIOACTIVITY}}{\text{EXPOSURE}}$

The bigger the BER, the greater the confidence that bioactivity will not occur in exposed consumers

Unilever

Points of Departure from NAMs can be protective
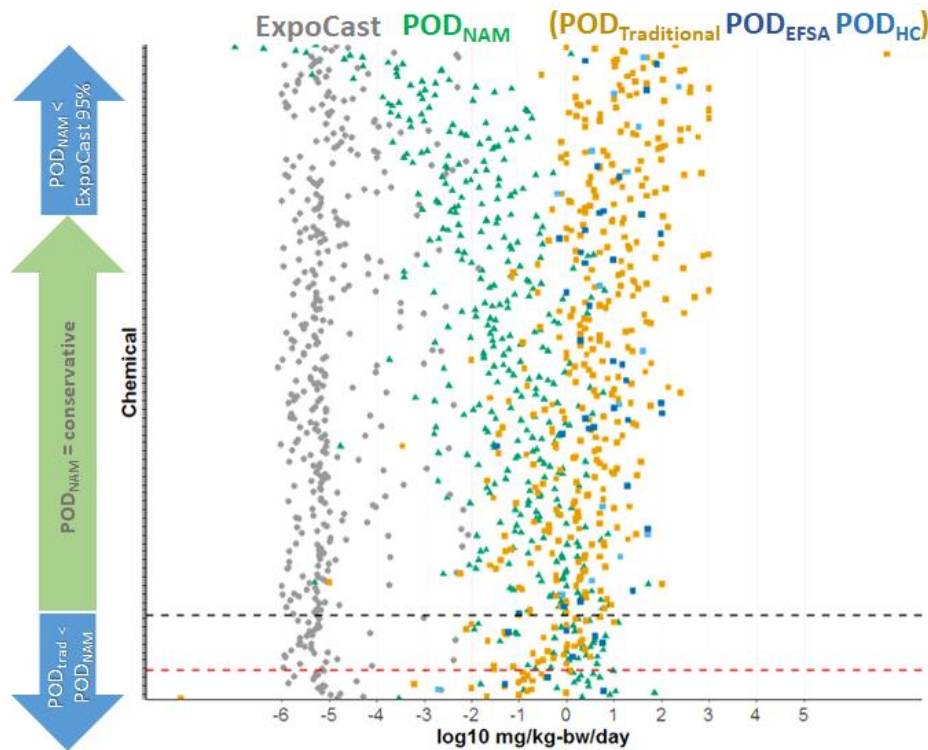
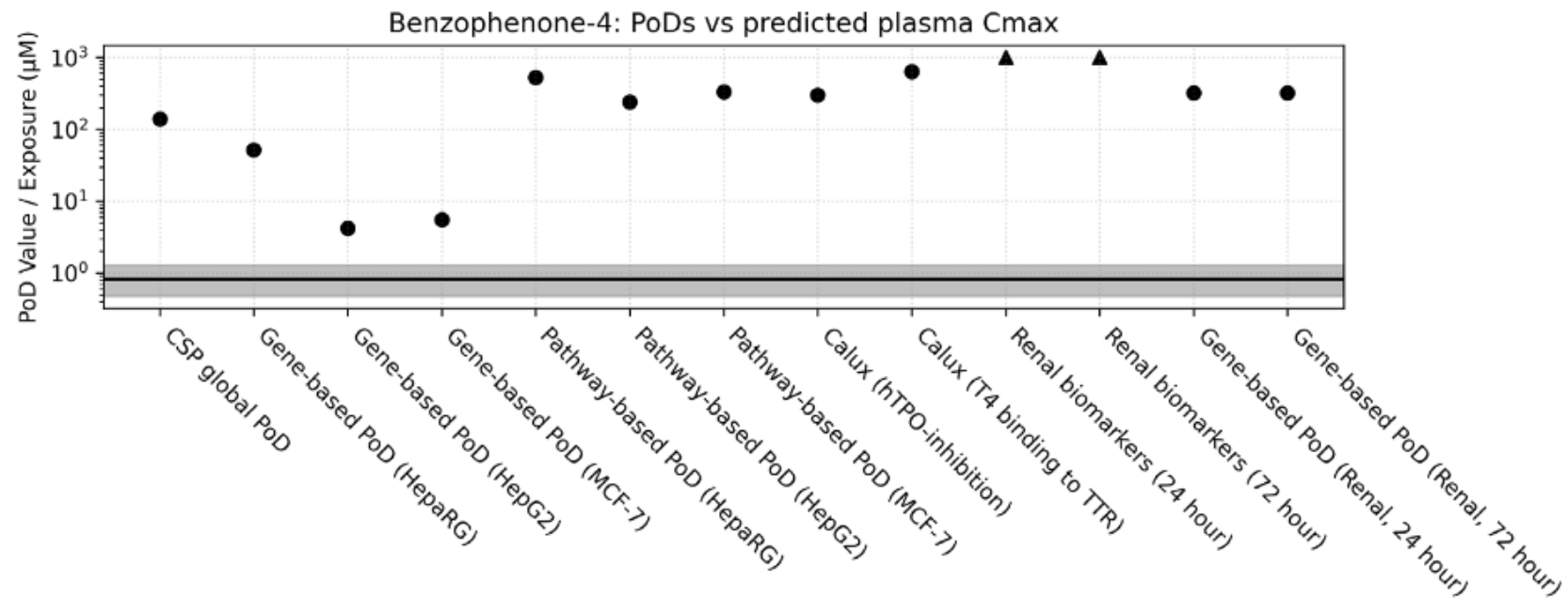Case Studies Demonstrating Application of Bioactivity as a Protective POD
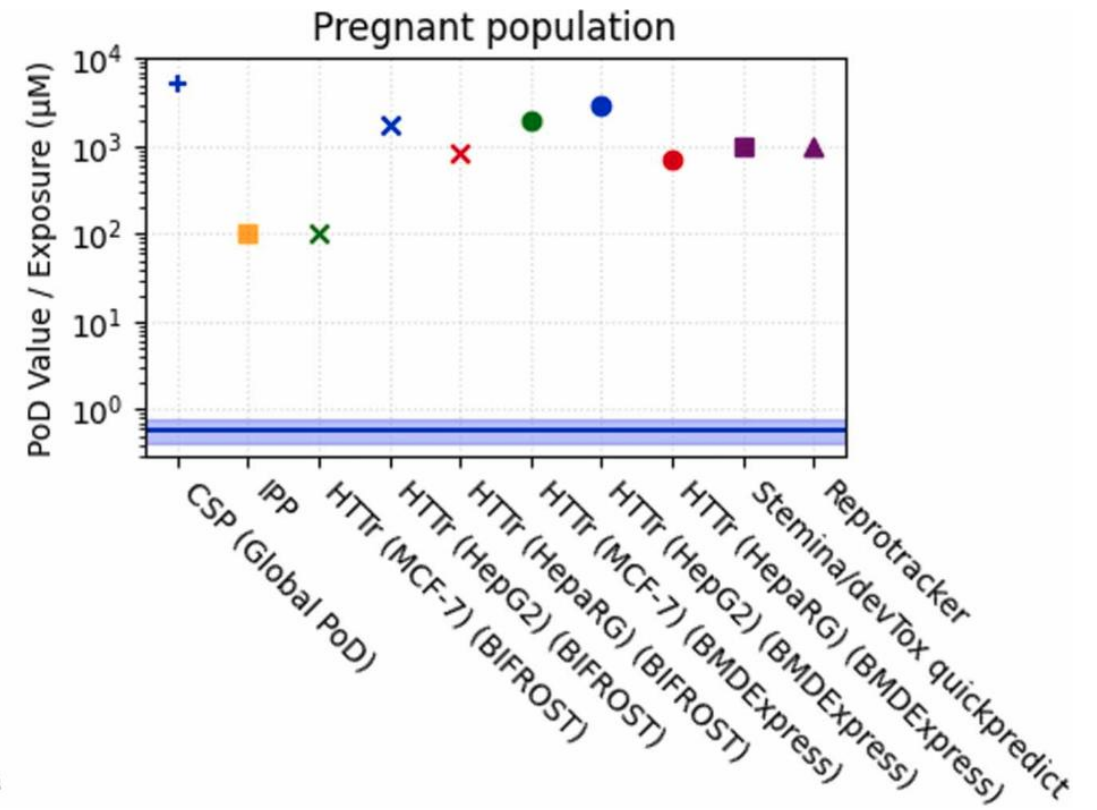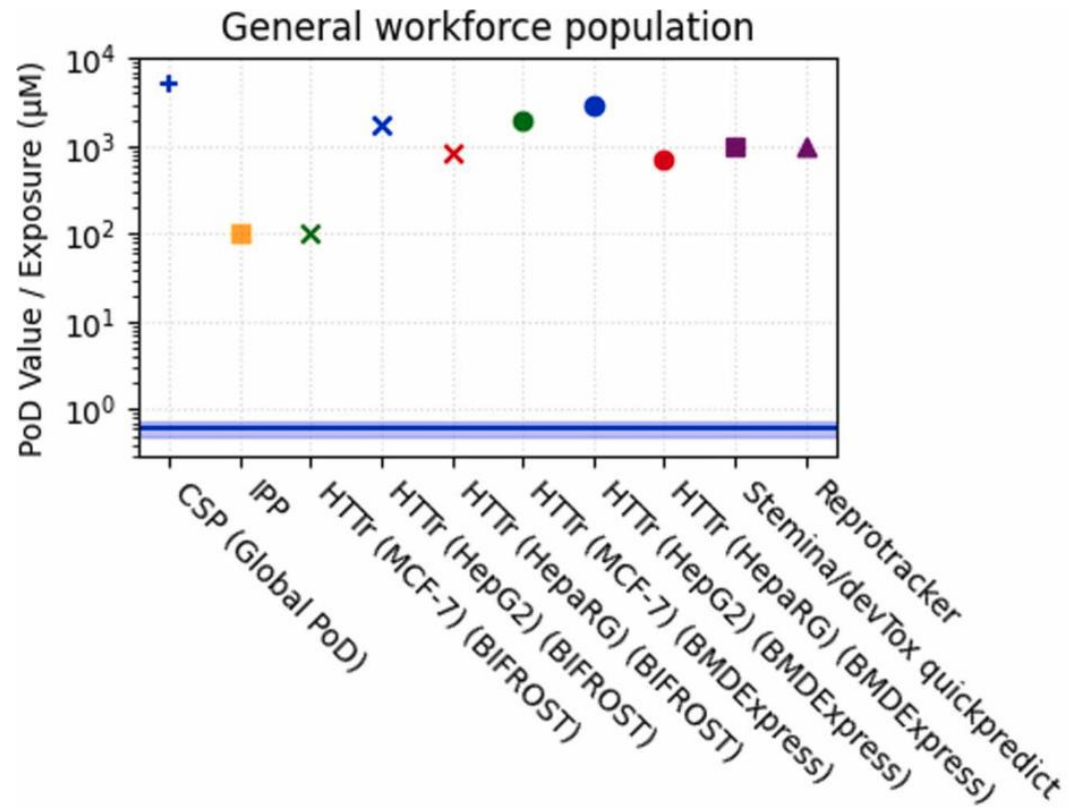Paul-Friedman et al., 2020
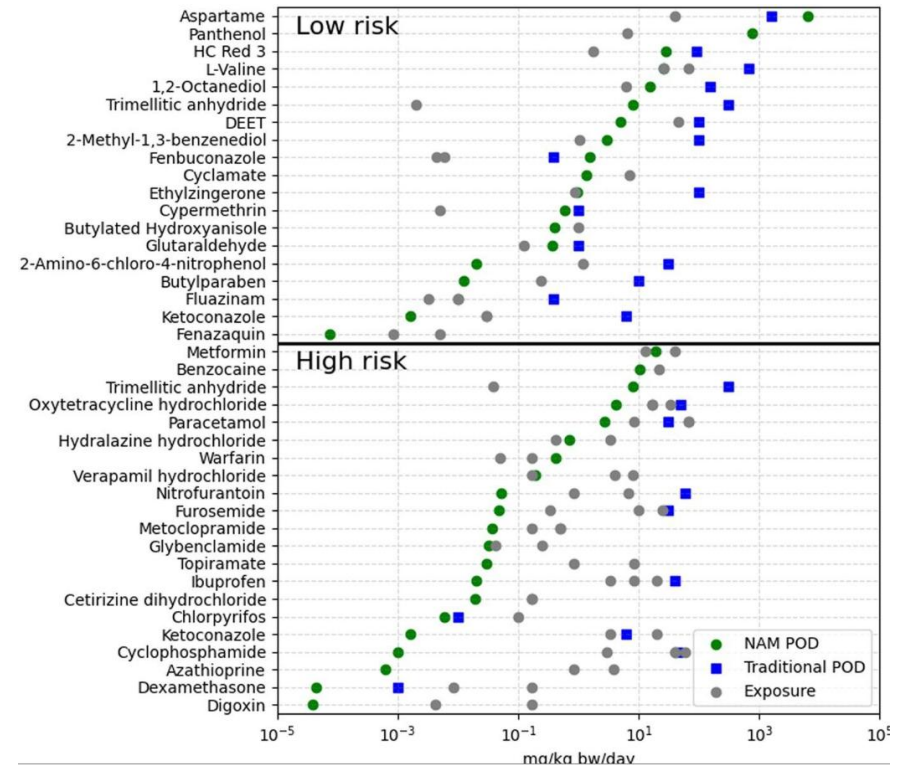
NAM-based assessments can be at least as protective as animal-based assessments
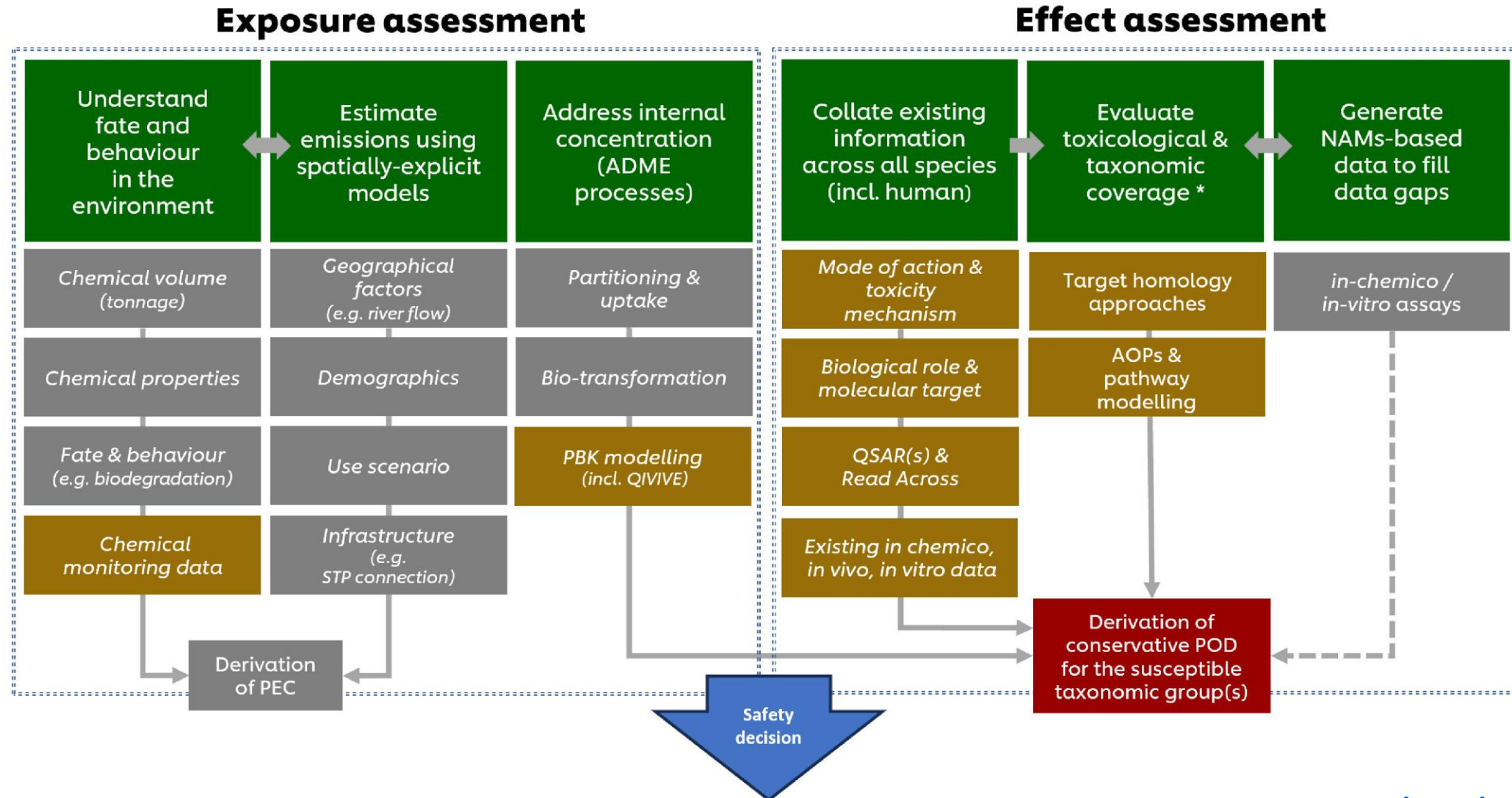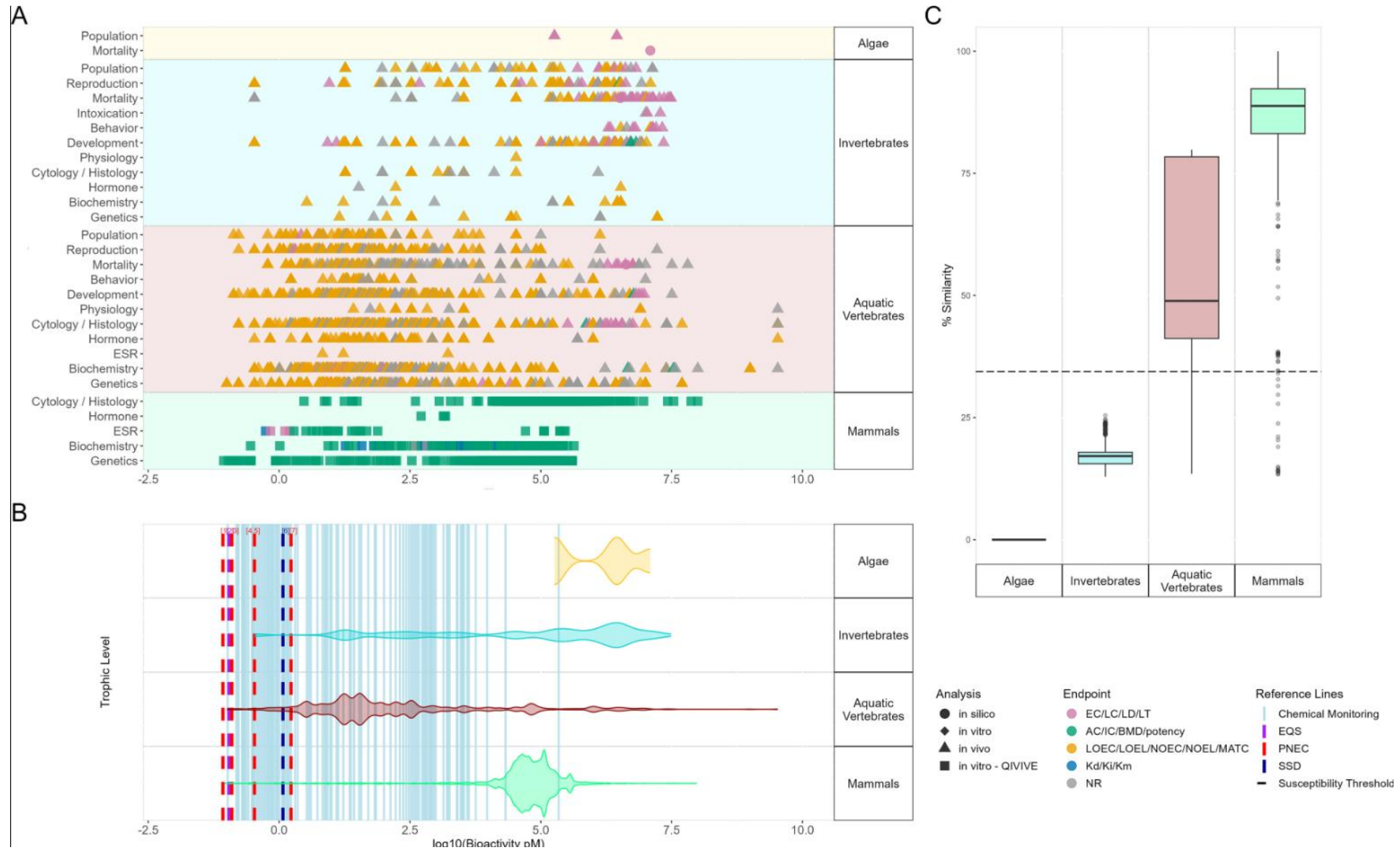Cable et al., 2024 (Toxicological Sciences)

Consumer Safety

Benzophenone-4: PoDs vs predicted plasma Cmax

Baltazar et al., 2025

Occupational Safety

Wood et al., 2024

Environmental Safety Framework

Rivetti et al., 2025

Rivetti et al., 2025

Summary

1. Good quality exposure data unlock the potential for protective NAM-based safety decision making

2. The same principles can be applied to consumer, occupation and environmental safety assessment

3. Closer alignment of human and environmental safety workflows will bring scientific and practical advantages, and challenges

Thank you

Unilever: Matt Dent, Alberto Locca, Alistair Middleton, Andy White, Ans Punt, Beate Nicol, Bruno Campos, Claire Peart, Claudia Rivetti, Dawei Tang, Danilo Basili, Gavin Maxwell, Geoff Hodges, Gopal Pawar, Iris Muller, Jacob Collins, Jade Houghton, Katarzyna Pryzbylak, Katy Wilson, Kathryn Wolton, Leonardo Contreas, Magdalena Sawicka, Maria Baltazar, Maria Blanco Rubio, Matt Dent, Patrik Engi, Paul Carmichael, Renato Ivan de Ávila, Sandrine Spriggs, Sophie Malcomber, Tym Pietrenko and many more

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