Progress applying the Principles of NGRA to UV filters

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Outline

- *The ICCR Principles of Next Generation Risk Assessment (NGRA)*
- *From Principles to Application*
- *Example: Benzophenone-4*
- *Future opportunities*

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Unilever

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

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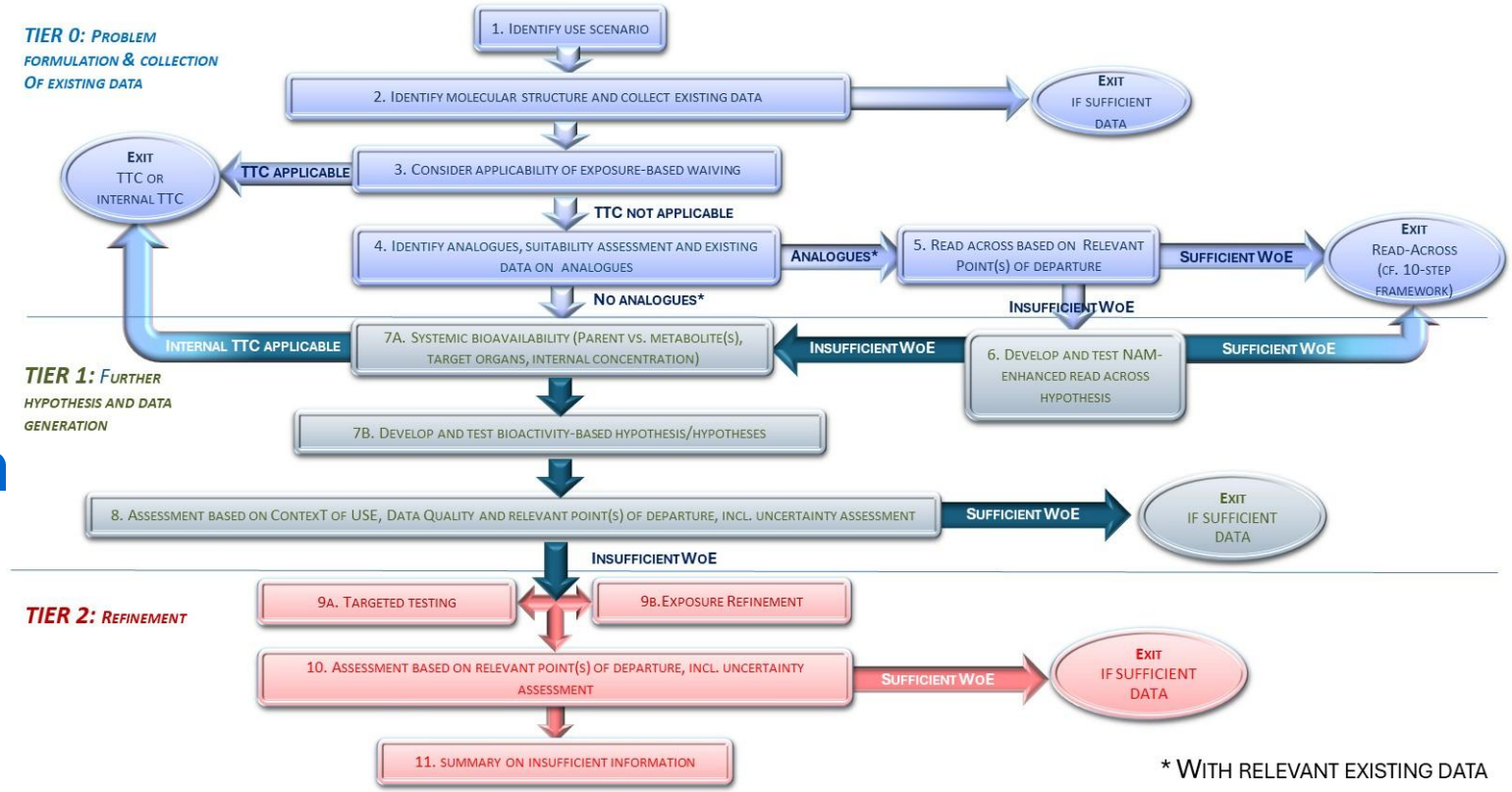
https://doi.org/10.1016/j.comtox.2018.06.001

Fundamental Enablers:

1. Start with exposure, not hazard
2. Use a tiered approach



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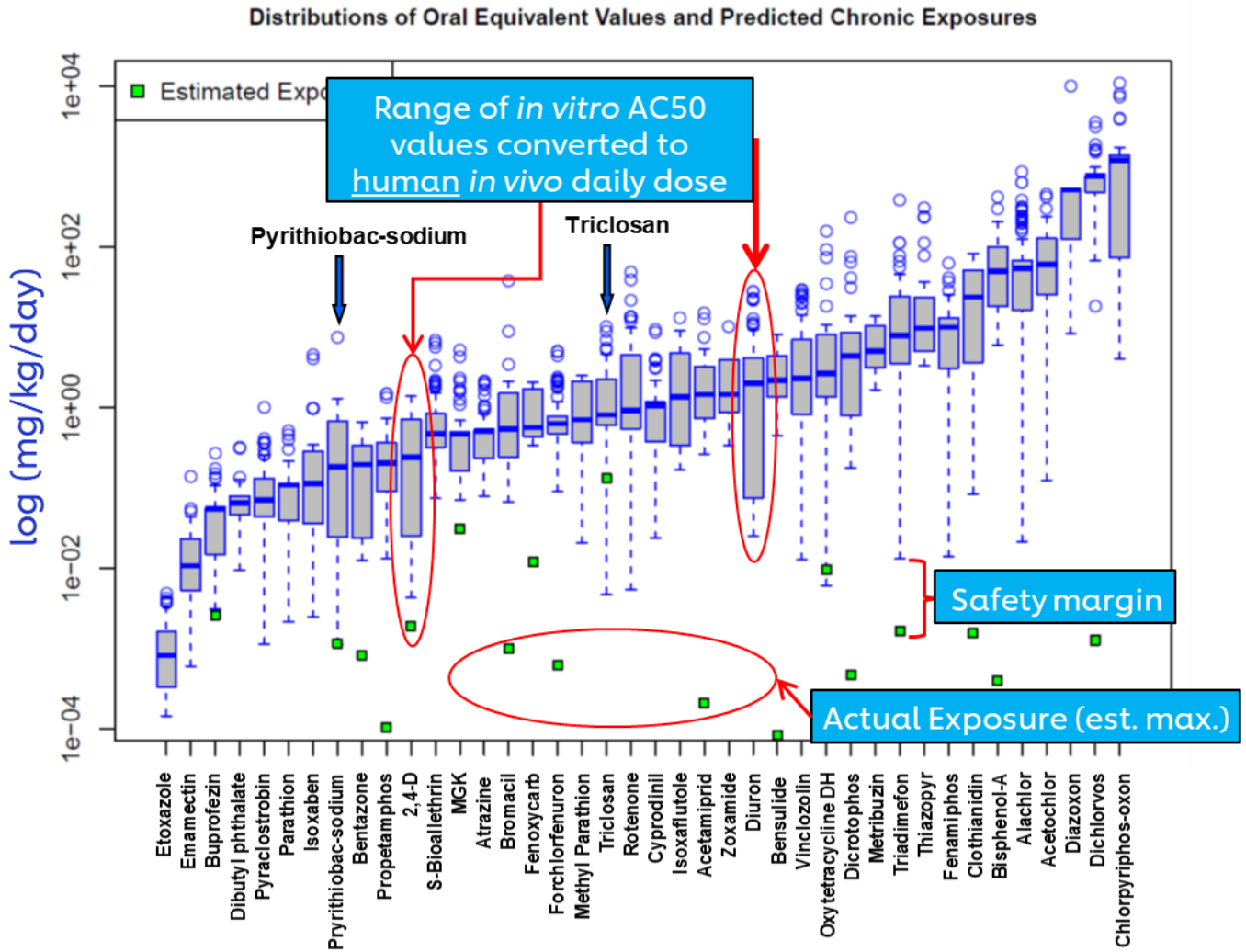
ICCS | INTERNATIONAL COLLABORATION ON COSMETICS SAFETY

From Principles to Application

If there is **no** bioactivity observed at consumer-relevant concentrations, there can be no adverse health effects.

If there **is** bioactivity observed at consumer-relevant concentrations, follow up testing is required to establish if that could result in an adverse effect.

This assessment does not try to **predict** the results of high dose toxicology studies in animals.



Graph from Rusty Thomas, with thanks. Rotroff et al., (2010) Toxicol Sci 117:348-358

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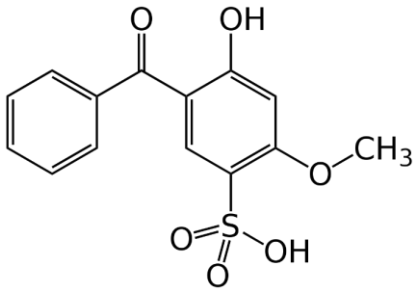
Benzophenone-4 (BP-4) case study: Introduction

- In 2019, the European Commission defined a list of 28 cosmetic ingredients with potential endocrine activity
- BP-4 is one of the 28 chemicals for which the call for data took place
- BP-4 is an **UV-filter ingredient used in sunscreen cosmetics**



CAS No. 4065-45-6; EC No. 223-772-2; sulisobenzone; 2-Hydroxy-4-methoxybenzophenone-5-sulphonic acid)

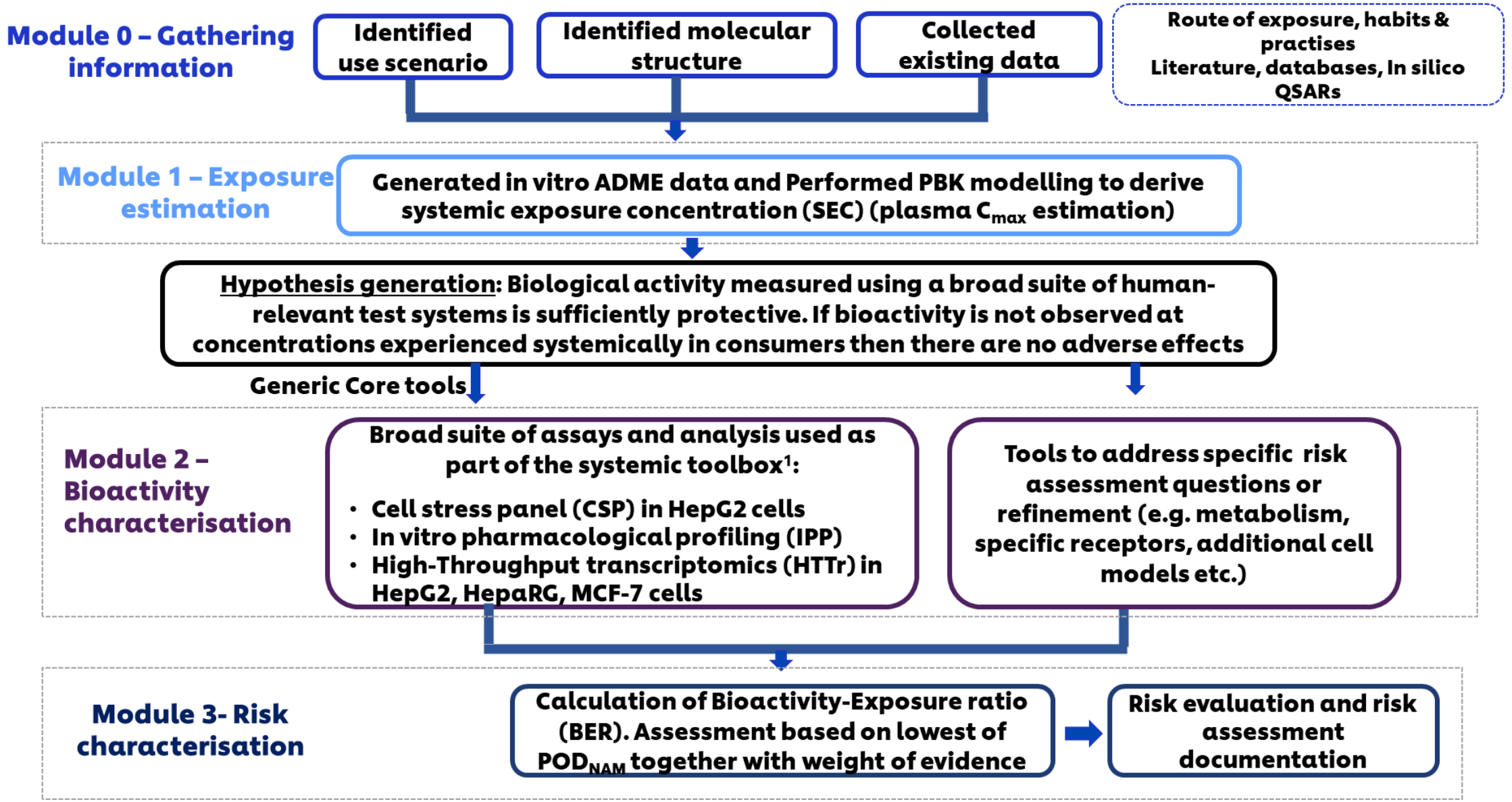
Objective of the case study:

- **To assess whether a tiered NGRA approach is sufficiently protective and also useful to answer a real-life question**
- For the purposes of this exercise, it has been assumed that **no *in vivo* animal data exist on the ingredient and no read-across**
- Focus on **systemic toxicity** (excluding genetic toxicity or DART) **using NAMs**

Is Benzophenone-4 safe in a sunscreen product at the maximum approved level of 5%?

Baltazar et al., 2025

The Tiered Approach



Module 1: plasma Cmax prediction for the population

- **Mean population plasma Cmax of 0.9 µM** (5th and 95th percentile of 0.4 and **1.24** µM, respectively)
- The influx rates of OAT1, OAT2, and OAT3 were higher than the efflux rates of BCRP and MRP4
- Limited distribution organs

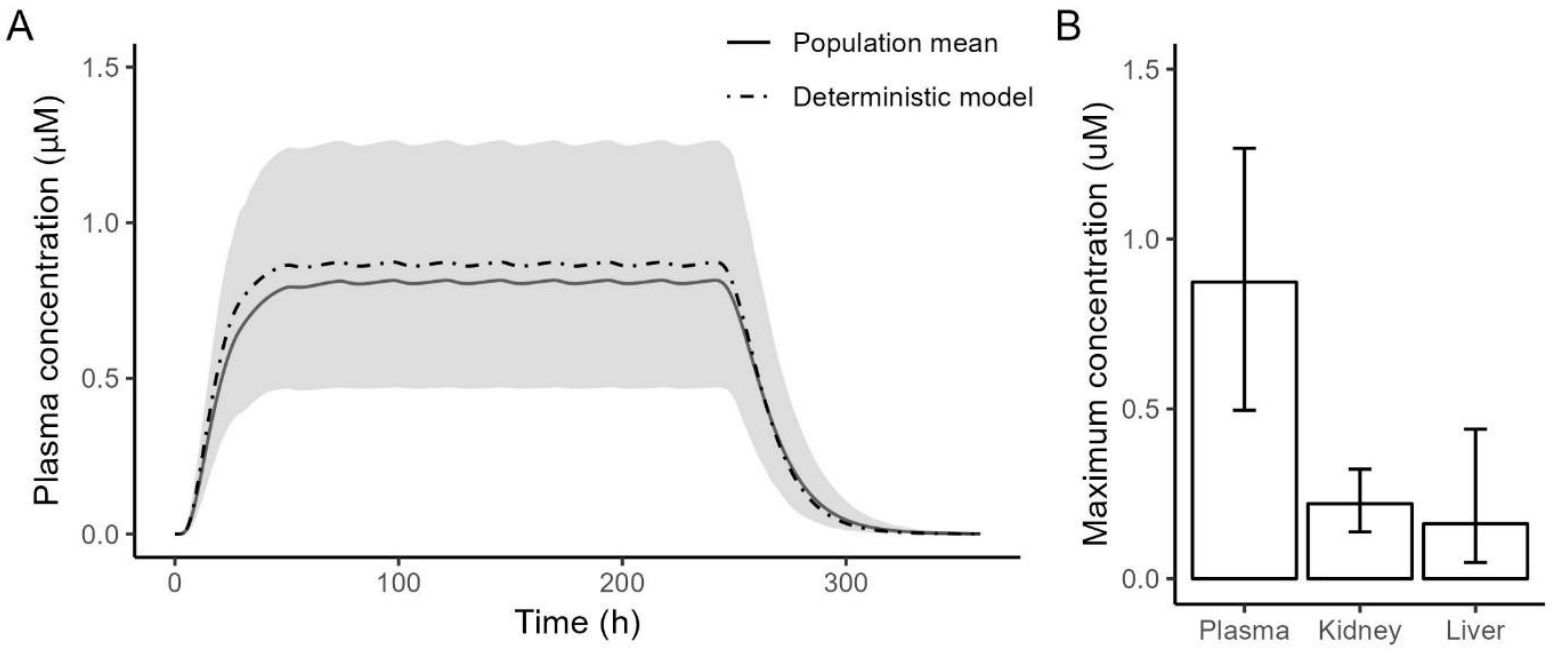


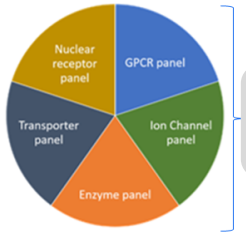
Figure. Population PBK simulation results (time course data and C_{max}) on benzophenone-4 concentrations in plasma after repeated exposure of body lotion 18g/day, i.e., 9g two times per day for a period of 10 days, with 5% benzophenone-4, on the whole body.

Problem formulation after collating existing information and exposure estimation

Hypothesis	Testing strategy
• BP-4 could bind to estrogen receptor (VEGA in silico tool flagged a potential binding to estrogen receptor)	• *In vitro* CALUX® EATS (estrogenic, androgenic, thyroidogenic and steroidogenesis)
• Cell models previously tested (HepG2, HepaRG and MCF-7) might lack the transporters involved in BP-4 organ distribution • Potential underestimation of bioactivity • BP-4 distribution to only kidney and liver	• Literature review of cell lines expressing the key transporters • Addition of a primary proximal tubule cell model to evaluate BP-4 bioactivity.
• Absence of in silico alerts ≠ no toxicity	• Test a systemic toolbox using non targeted (transcriptomics, cell stress panel) & targeted NAMs (in vitro pharmacological profiling)

BIOACTIVITY

In vitro pharmacological profiling



79 targets

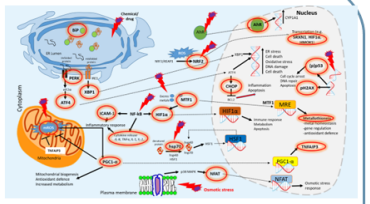

PERSPECTIVES
Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

eurofins | Cerep

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

Cell stress panel (CSP)

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

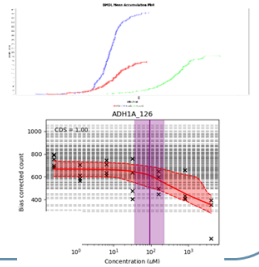


Hatherell et al. 2020. Toxicol Sci 176(1): 11-33

Image kindly provided by Paul Walker (Cyprotex)

High-Throughput transcriptomics (HTTr)

- TempO-seq technology – full gene panel
- 24hr exposure
- 7 concentrations
- HepG2, MCF7, HepaRG
- Dose-response analysis using BMDExpress2 and BIFROST model



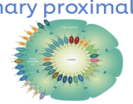
Reynolds et al. 2020. Comp Tox 16: 100138
Baltazar et al. 2020. Toxicol Sci 176(1): 236–252

EATS activity: estrogenic, androgenic, thyroidogenic and steroidogenesis

- CALUX bioassays to measure transcriptional activation and binding assays:
  - U2-OS incorporating the firefly luciferase reporter gene coupled to Responsive Elements (REs)
  - ERα, AR, TTR-TRβ- and hTPO
- In vitro H295R Steroidogenesis Assay (H295R) utilises human adenocarcinoma cell line NCI-H295R. Quantification of 17β-estradiol and Testosterone is performed using the AR CALUX and ERα CALUX bioassays
- 12 concentrations. Calculation of AC50, LOEC and NOEC

Renal Toxicity

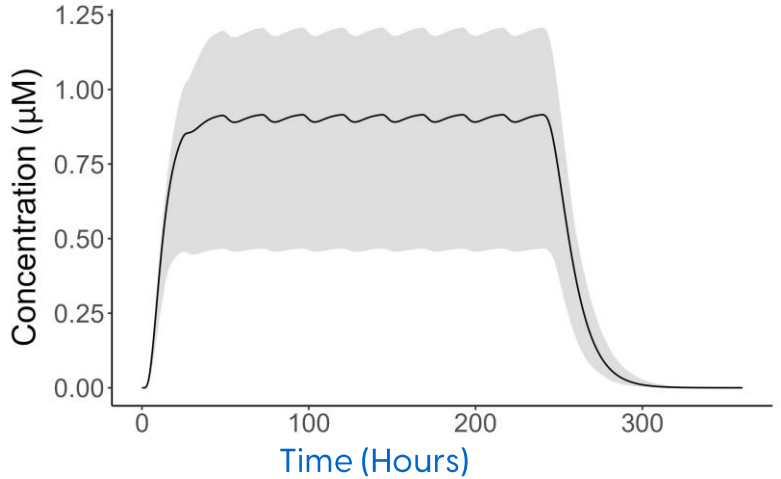
Renal biomarkers (3 donors, duplicate per donor), 8 concentrations, 24h and 72h timepoints in primary proximal tubule cell:



- KIM-1, NGAL, Clusterin,
- TEER (Day 0 and Day 3), ATP, LDH
- Toxicogenomics (3 donors, 2 duplicates per donor), 8 concentrations, 24h and 72h timepoints
- Omeprazole and cisplatin added as benchmarks/positive controls

Piyush Bajaj et al. 2020. Toxicology. 442, 152535

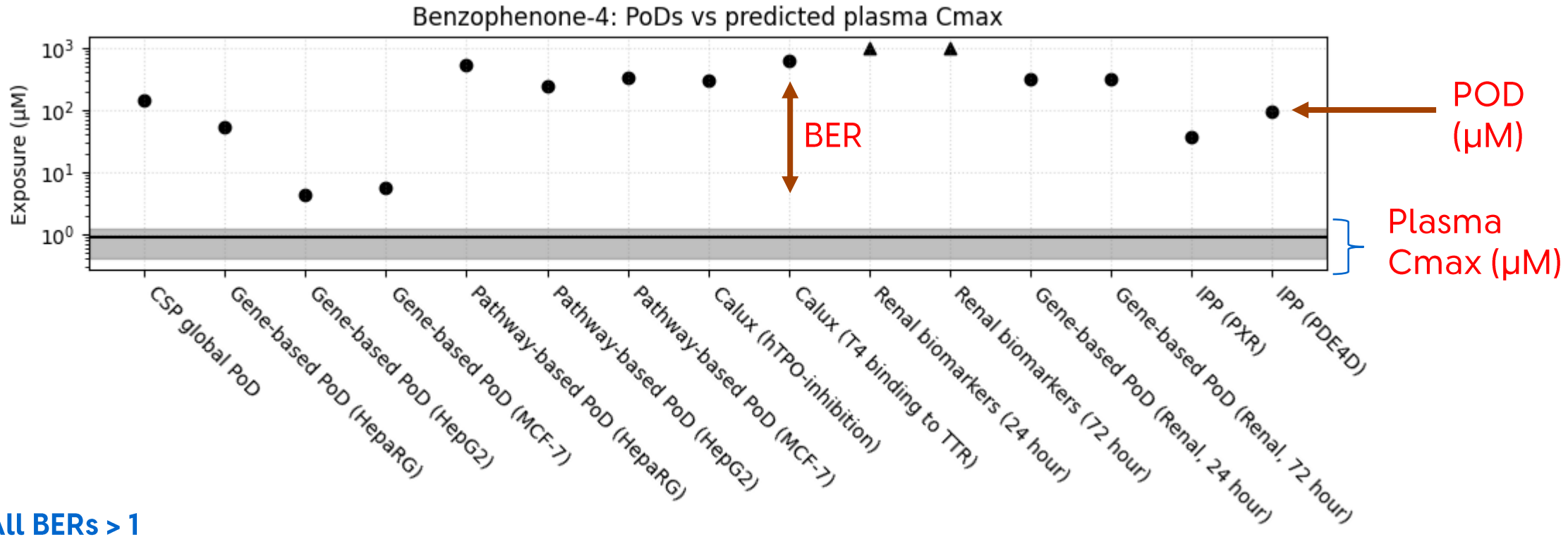
EXPOSURE



BIOACTIVITY EXPOSURE RATIO = $\frac{\text{BIOACTIVITY (µM)}}{\text{EXPOSURE (µM)}}$

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

Bioactivity: exposure ratio calculation: BERs ranging from 3.4-508



- **All BERs > 1**
- **Lowest BER (3.4):** PODs was obtained from HTTr in HepG2 cells when considering lowest gene change (POD of 4.2 µM). BER obtained from pathway level POD was 193.
- **BER of 508:** POD_{NAM} derived from the Calux assay (T4 binding to TTR).

Current Focus

Actively including NAMs in submissions to regulatory authorities worldwide (alongside historical animal data where available)

Building confidence in the application of NGRA in different decision contexts (cosmetics, industrial chemicals, foods, homecare)

ELSEVIER

Current Opinion in Toxicology

Available online 23 June 2026, 100601

In Press, Journal Pre-proof What's this?

NGRA in Cosmetic Safety: Moving From the Frameworks and Case Studies to Wide Adoption

Ivan Rusyn [1], Weihsueh A. Chiu [1], Matthew Dent [2], Alistair Middleton [2]

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Concluding remarks

1. The NGRA principles provide the means to apply NAMs to answer relevant safety and regulatory questions

2. Tiered, exposure-led frameworks means we can do this today

3. NAMs are being applied globally

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