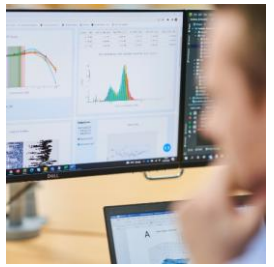
Advancing the Application of Next Generation Science to Make Safety Decisions

DR Maria Baltazar,

Safety Science Capability Lead Unilever Safety, Environmental & Regulatory Science , UK

SERS
Safety, Environmental & Regulatory Science



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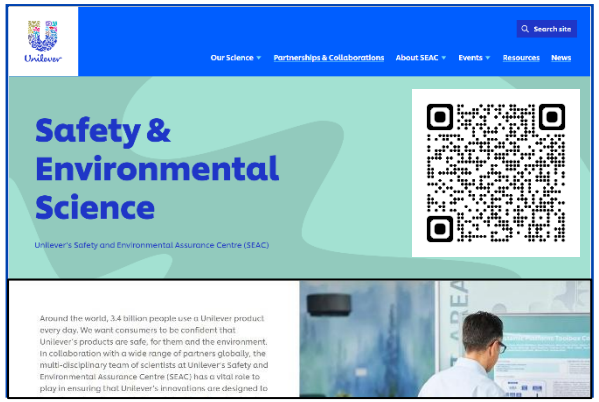
Outline

- Introduction to Next generation risk assessment (NGRA)
- Unilever approach to developing an early tier NAM-systemic toolbox and workflow
- Application of NGRA principles to case studies



Our Purpose is to use leading-edge Science & Data to:

1 **Protect** People & the Environment from harm

2 **Enable** product Innovation, De-risking & Compliance

3 **Pioneer** industry & regulatory application of New Approaches, in partnership with other change leaders

SERS
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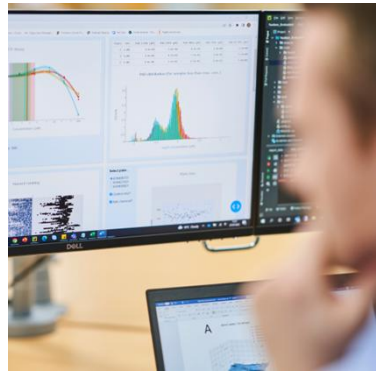
Pioneers & Trusted Partners in Regulatory Science, co-creating the Future for Superior, Safe & Sustainable Products



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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:

Consumer Exposure

Potential hazards of the ingredients

Risk Assessment

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https://health.ec.europa.eu/latest-updates/sccs-notes-guidance-testing-cosmetic-ingredients-and-their-safety-evaluation-12th-revision-2023-05-16_en

Introduction to Next generation risk assessment (NGRA)

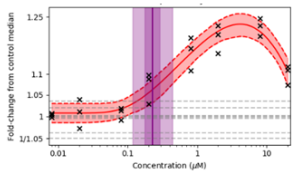
NGRA is defined as an exposure-led, hypothesis-driven risk assessment approach that integrates New Approach Methodologies (NAMs) to assure safety without the use of animal testing[1]

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

An approach to Next Generation Risk Assessment – Protection of human health

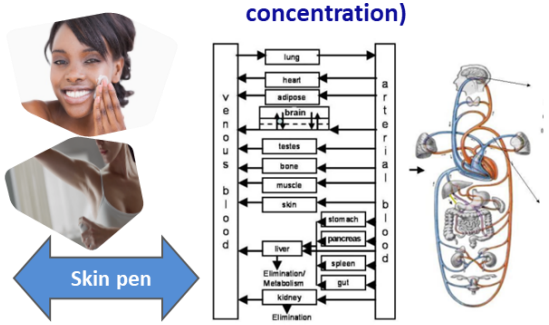
Point of departure (POD) derived from concentration-response data



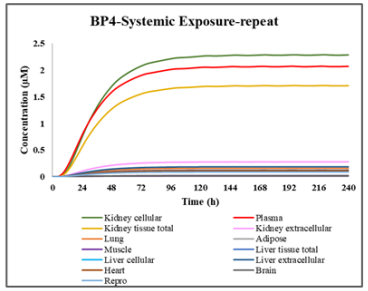
Systemic toolbox of assays (NAMs) which cover a broad biological space – **measurements of bioactivity**

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

Exposure models (PBK, free/total concentration)



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



Calculation of Bioactivity exposure ratio (BER)

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

If there is <u>no bioactivity</u> observed at consumer-relevant concentrations, there can be <u>no adverse health effects.</u>

If there <u>is bioactivity</u> observed at consumer-relevant concentrations -> <u>is it adverse</u>?

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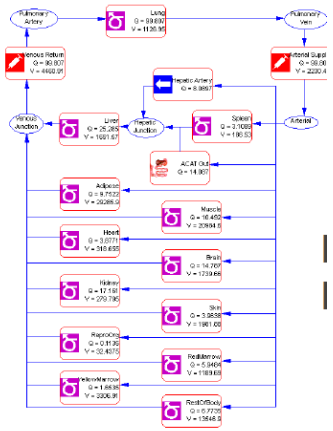
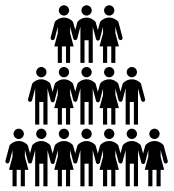
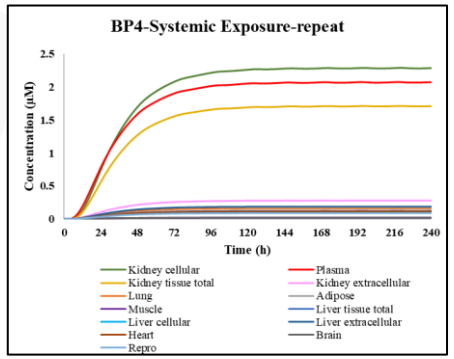
Our Key NAMs

Internal exposure - PBK modelling

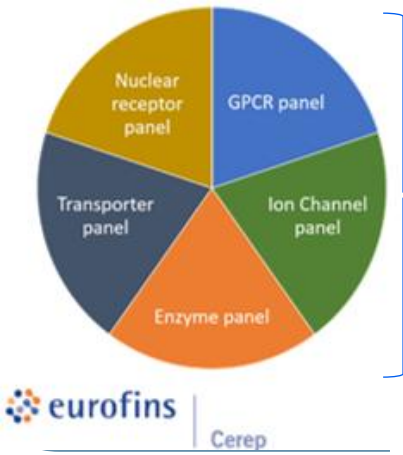






Moxon TE et al., 2020. Toxicology In Vitro, 63, 104746

In vitro pharmacological profiling

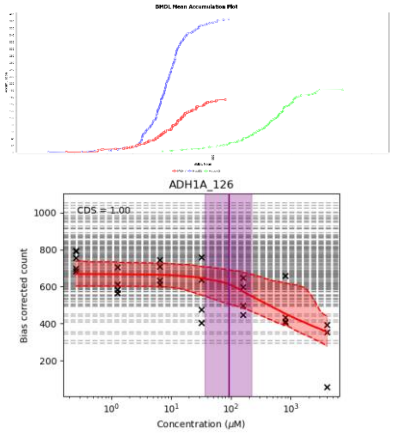


~79 targets

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



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Cell stress panel (CSP)

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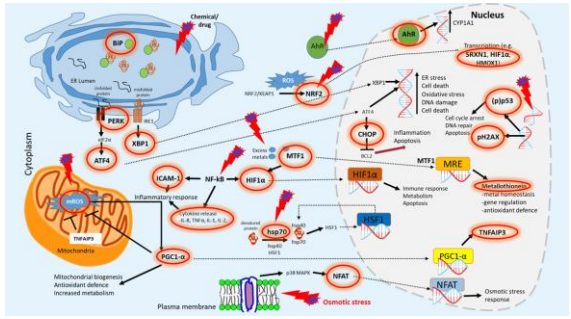
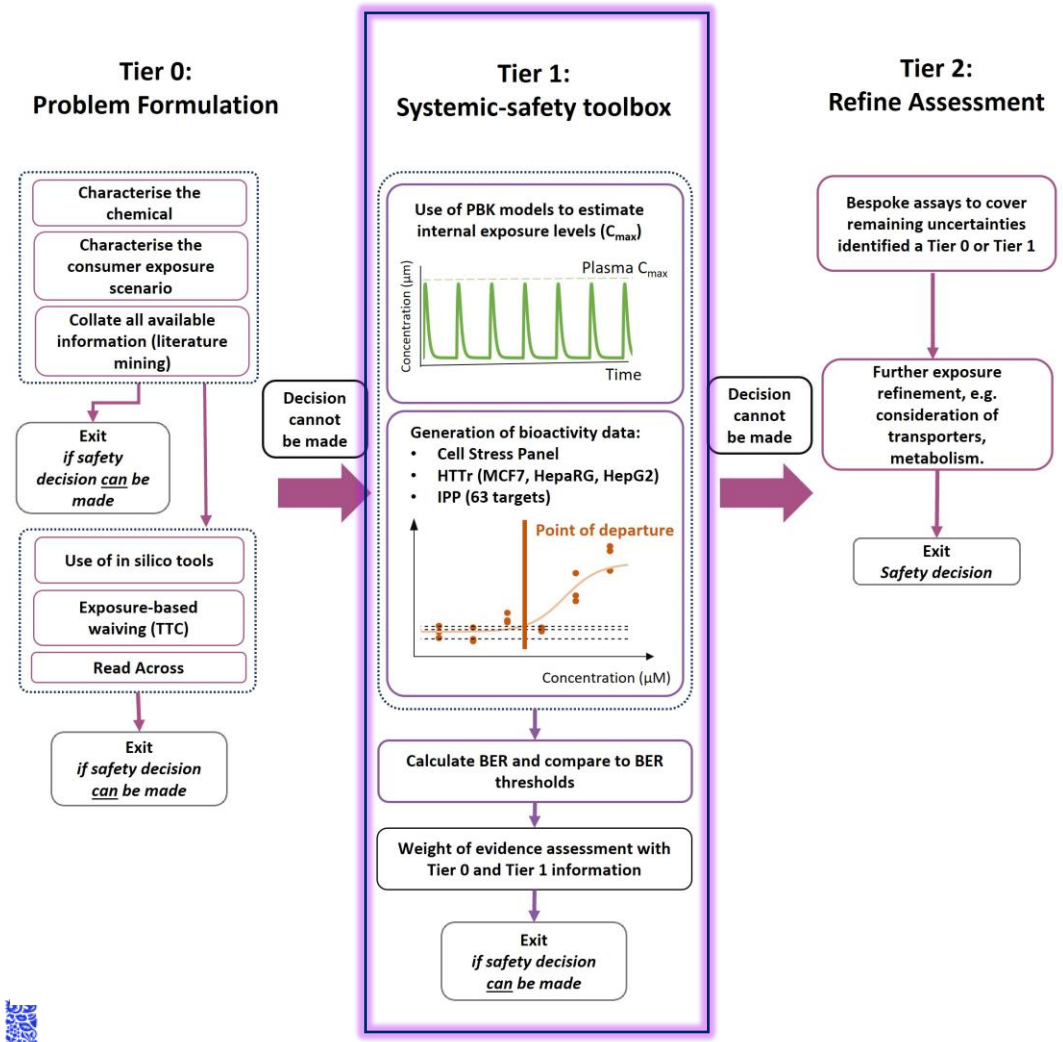


Image kindly provided by Paul Walker (Cyprotex)

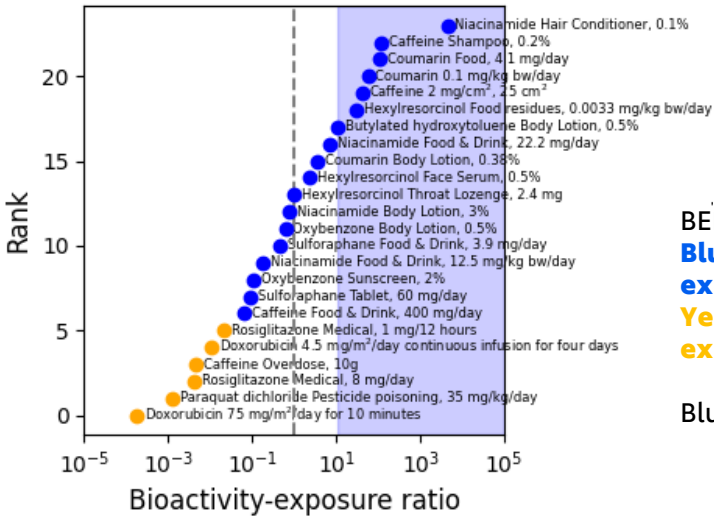
Hatherell et al. 2020. Toxicol Sci 176(1): 11-33

Cable S et al., (2024). https://doi.org/10.1093/toxsci/kfae159; Middleton et al., 2022. https://doi.org/10.1093/toxsci/kfac068

Our approach for systemic toxicity – A NAM toolbox and workflow



NAM Systemic toolbox provides similar level of protection as traditional approaches for a total of 48 chemicals and 100 chemical exposure scenario



BER=lowest POD/Plasma Cmax
Blue: low risk chemical-exposure scenario
Yellow: high risk chemical-exposure scenario

Blue shaded region BER> 11

Cable S et al., (2024). https://doi.org/10.1093/toxsci/kfae159; Middleton et al., 2022. https://doi.org/10.1093/toxsci/kfac068

Making Safety Decisions for a Sunscreen Active Ingredient Using Next-Generation Risk Assessment: Benzophenone-4 Case Study

https://www.altex.org/index.php/altex/article/view/2934/version/2996

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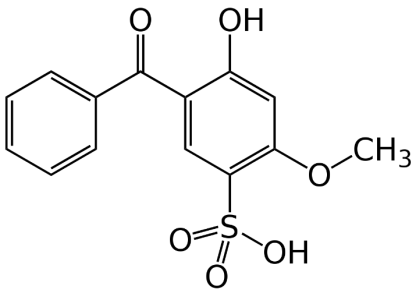
Benzophenone-4 (BP-4) case study: Introduction



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

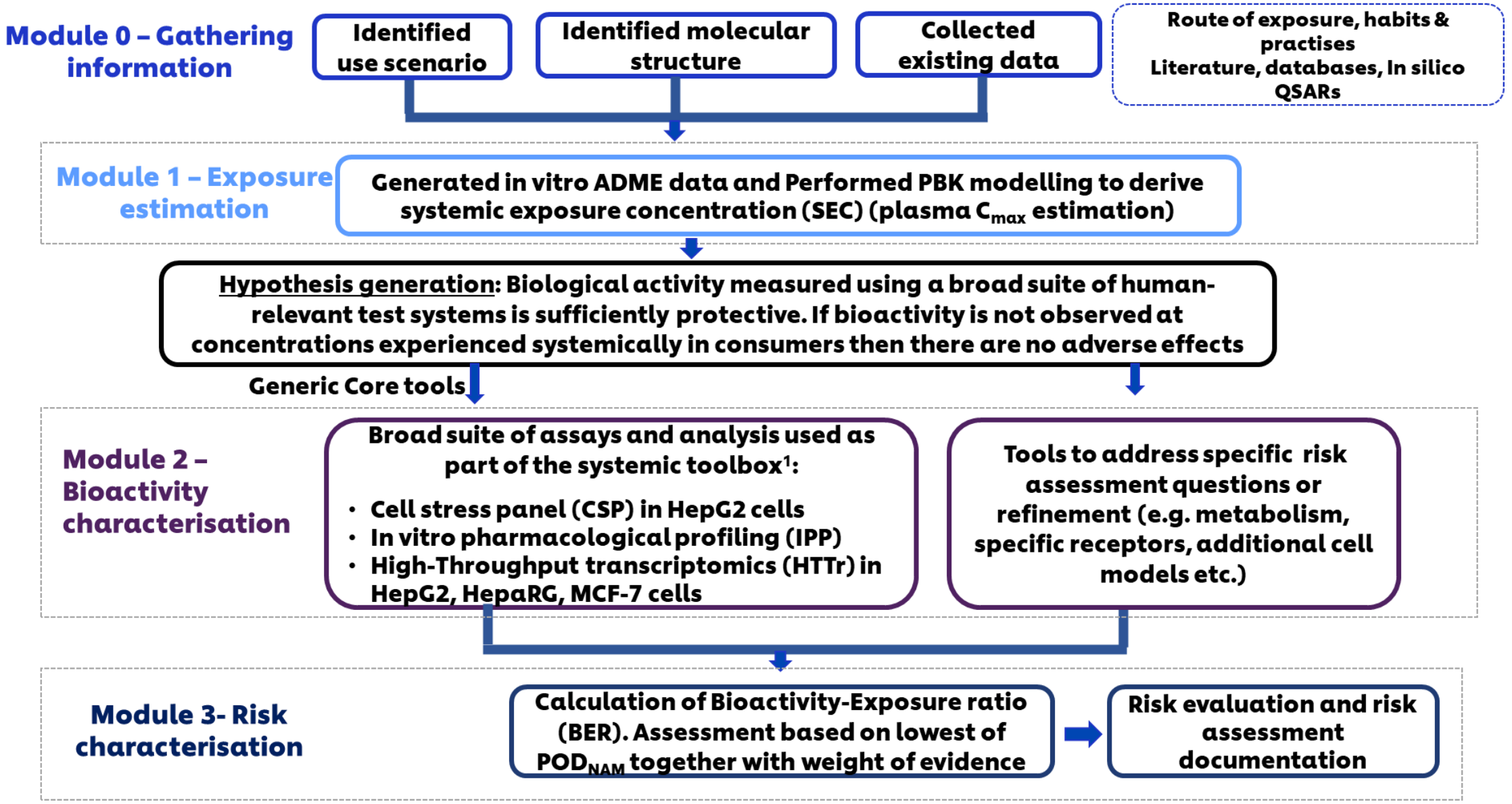
- In 2019, the European Commission defined a list of 28 cosmetic ingredients with potential endocrine activity
- BP-4 is one of the 28 chemicals for which the call for data took place
- BP-4 is an **UV-filter ingredient used in sunscreen cosmetics** to prevent sunburns or photodegradation by inhibiting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

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%?

Tiered approach to risk assessment



Module 0 – Gathering information

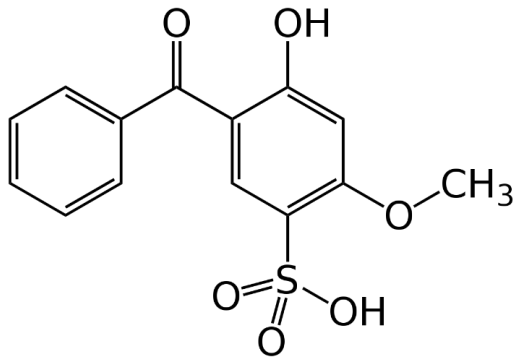
Identified use scenario

Identified molecular structure

Collected existing data

Route of exposure, habits & practises
Literature, databases, In silico QSARs

- **Tools used:** DEREK Nexus, METEOR Nexus, OECD Toolbox, TIMES, OPERA, VEGA



- **Results:**
  - **Benzophenone-4 did not trigger many alerts within the tools used**.
  - **Benzophenone-4 triggered one potential alert for estrogen receptor binding in the VEGA profiler**, however this was not consistent across other profilers that also assess estrogen receptor activity.

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COSMETICS EUROPE LRSS

Module 1: steps to estimate internal exposure

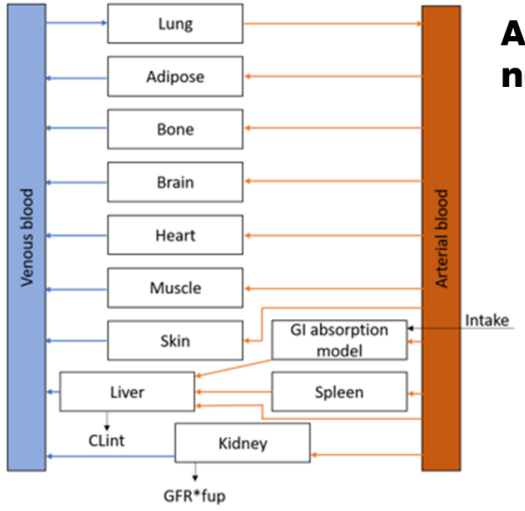
Exposure scenario (applied dose)

- 5% in Sunscreen product,
- 18g/day, two times, 9g/application (as per SCCS notes of guidance)
- On body and face 17500cm2 (total body area)

ADME data for model building

Core model input:

- Absorption (dermal in case of BP-4)
- Partition coefficients, fraction unbound, blood:plasma ratio
- Liver metabolism
- Passive renal excretion (glomerular filtration rate * fraction unbound)



Advanced input (when needed):

- PAMPA permeability
- Transporter kinetics transfected cell lines

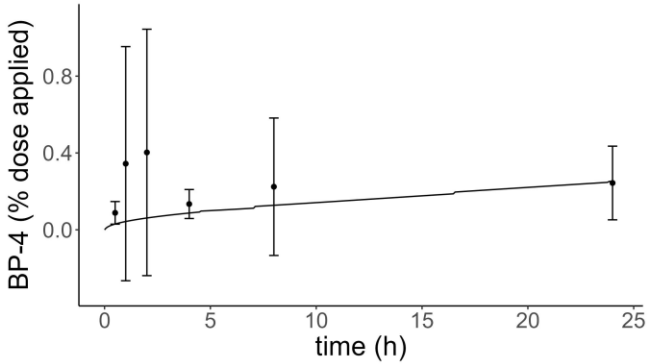
Population simulation

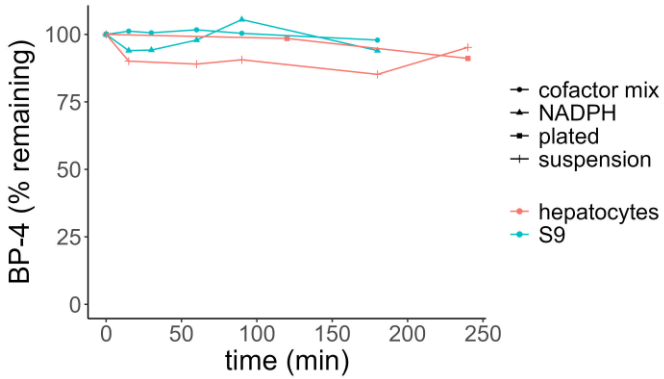
- Population of 50% females and 50% males, an age variation between 16 and 70 years, and a body weight range between 45-85 kg.

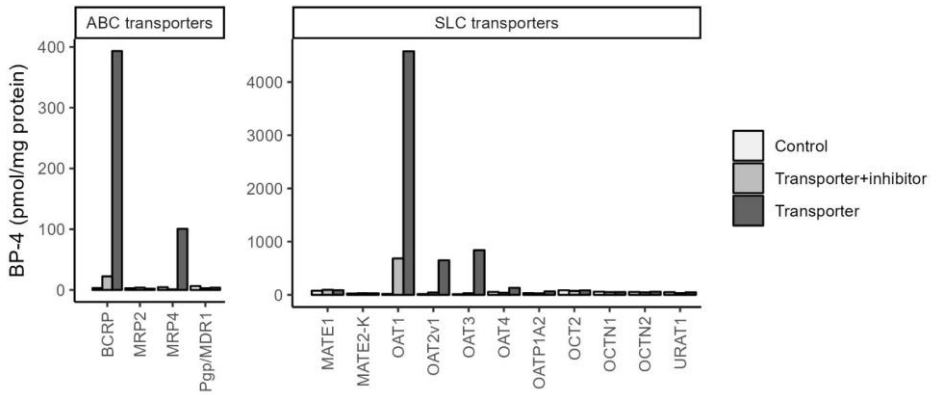
Software: GastroPlus 9.7

Module 1: Key ADME findings

- Limited dermal absorption (0.4%)
- Stable in primary human hepatocytes and S9 fraction (liver metabolism is negligible)
- BP-4 is a substrate of OAT1, OAT2, OAT3, BCRP, and MRP4 which indicates BP-4 is mainly secreted.
- In contrast, BP-4 was not found to be a substrate of transporters involved in reabsorption (movement from urine to blood).
- Limited membrane permeability (from PAMPA assay)







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, leading to substantial **concentrations within the liver (0.23 µM) and kidney (0.17 µM).**
- Limited distribution to any other organ

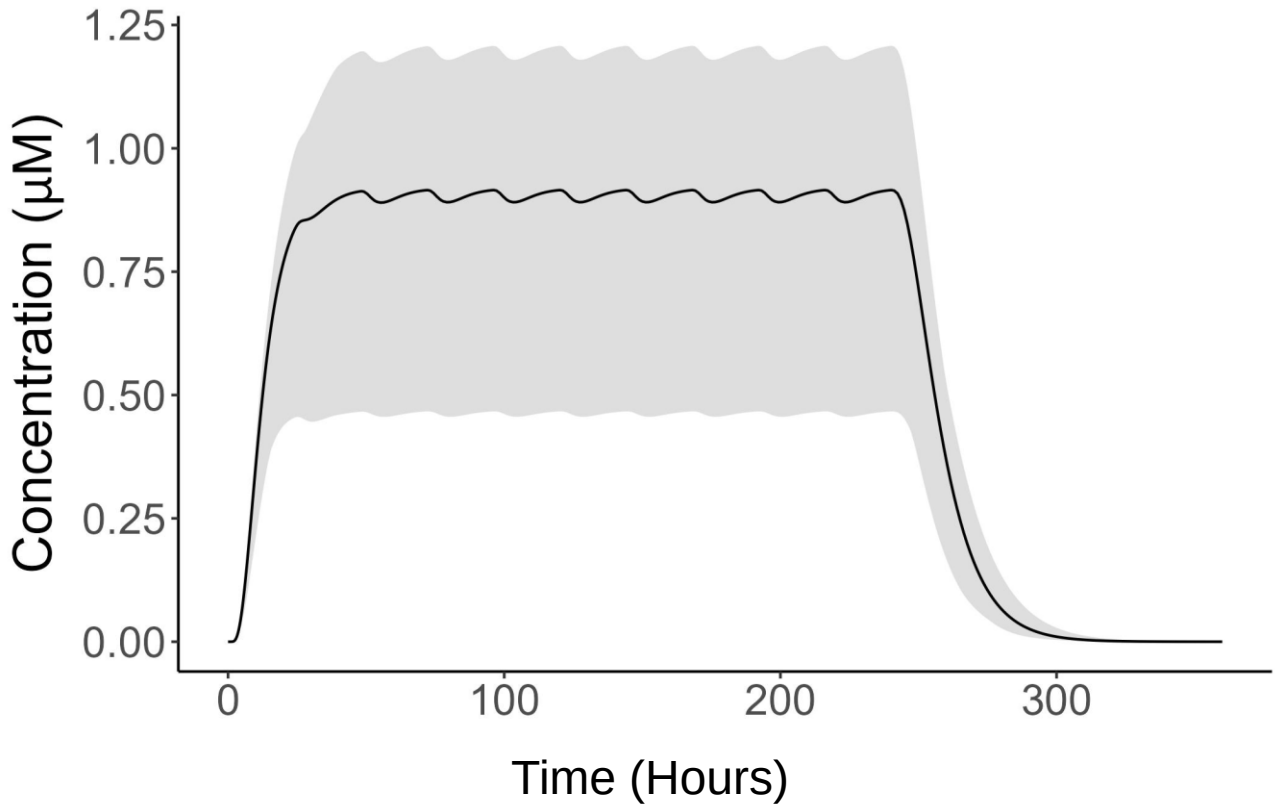


Figure. Population PBK simulation results (time course data and C_{max}) on benzophenone-4 concentrations in plasma after repeated exposure of body lotion 18g/day, i.e., 9g two times per day for a period of 10 days, with 5% benzophenone-4, on the whole body.

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)

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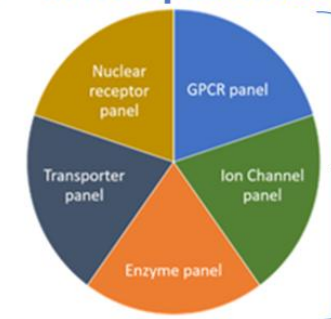
Module 2 – Bioactivity characterisation

Broad suite of assays and analysis used as part of the systemic toolbox:

- Cell stress panel (CSP) in HepG2 cells
- In vitro pharmacological profiling (IPP)
- High-Throughput transcriptomics (HTTr) in HepG2, HepaRG, MCF-7 cells

Tools to address specific risk assessment questions or refinement (e.g. metabolism, specific receptors, additional cell models etc.)

In vitro pharmacological profiling



~79 targets


PERSPECTIVES

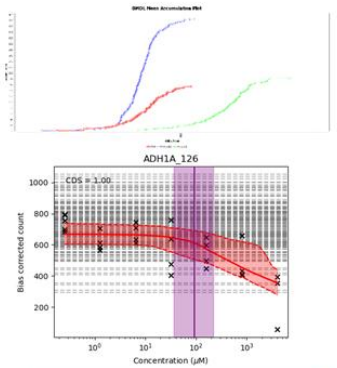
Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

eurofins | Cerep

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

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
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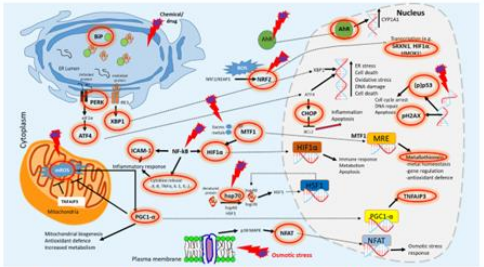

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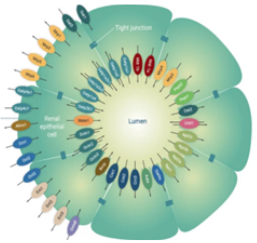
EATS activity: estrogenic, androgenic, thyroidogenic and steroidogenesis

- 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:



Newcells aProximate™ platform

- 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

Key Results & Deriving Points of Departure (PODs)

HTTr (HepG2, HepaRG, MCF7, PTC)

- Two approaches to calculating POD – BIFROST (gene level HepG2, 4.2 µM) and BMDL (pathway level HepG2 , 240 µM)
- Significantly lower bioactivity was detected in kidney cells (gene level: 320 µM). No pathways formed

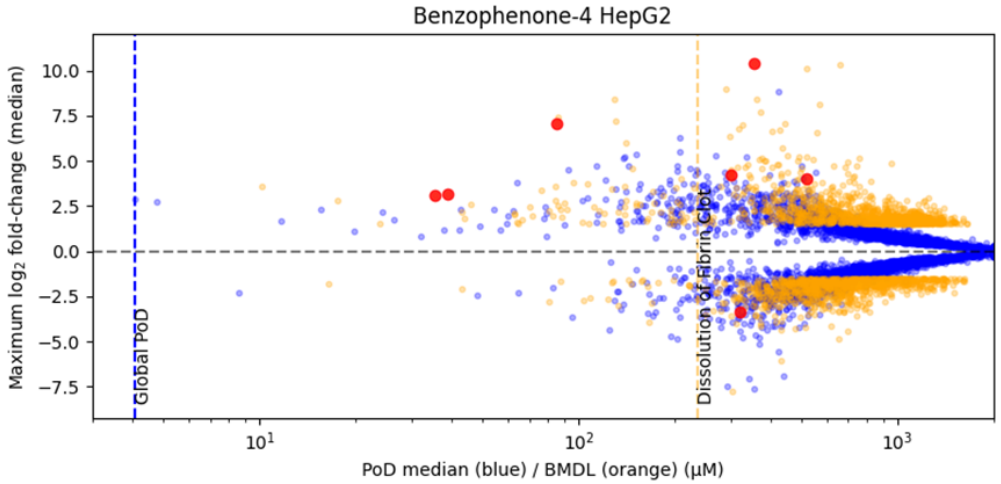
Cell Stress Panel

- Global POD_{NAM} = 140 µM

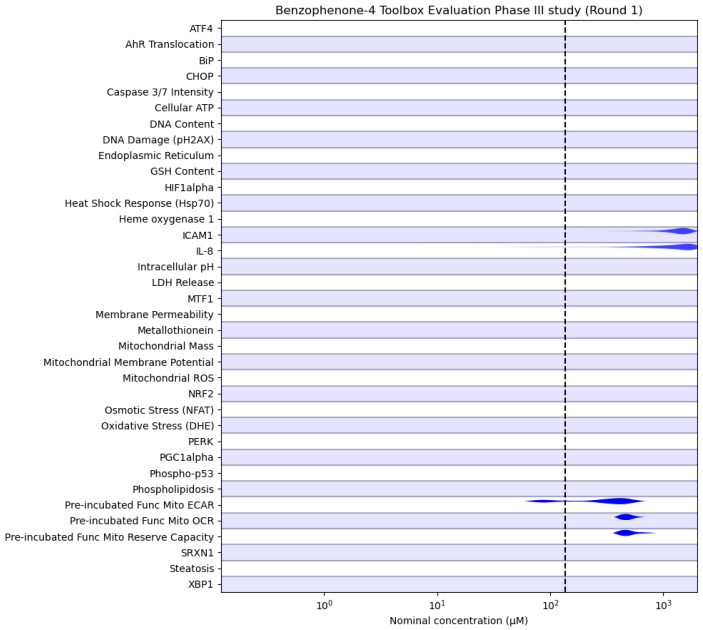
In vitro Pharmacological profiling

- Tested up to 10 µM
- ~83 targets compiled by Cosmetics Europe Safety pharmacology WG
- No hits

HTTr



Cell Stress Panel



Key Results & Deriving Points of Departure (PoDs)

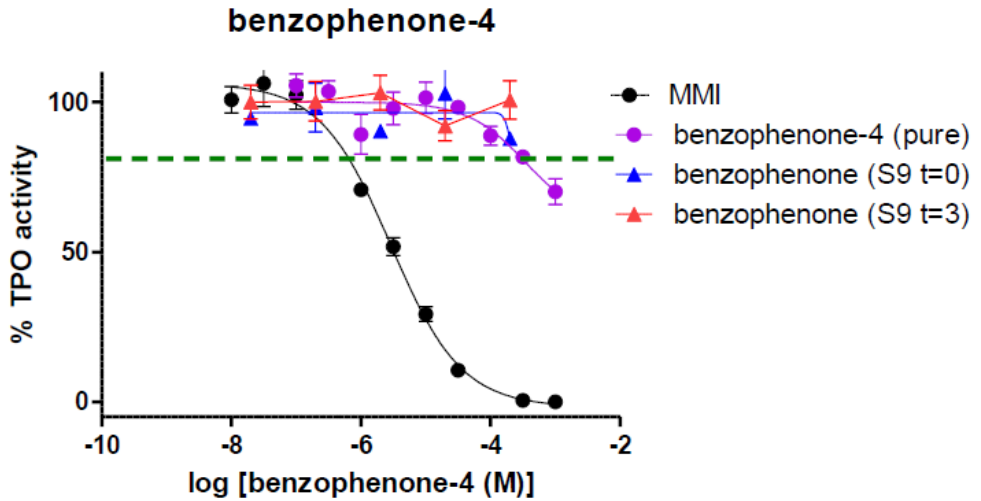
Calux assays

- No agonism or antagonism of ER, AR or TR and no effect on production of oestrogens or androgens ±S9
- Activity towards hTPO and TTR was found at high concentrations (LOEC= 300-600 µM).

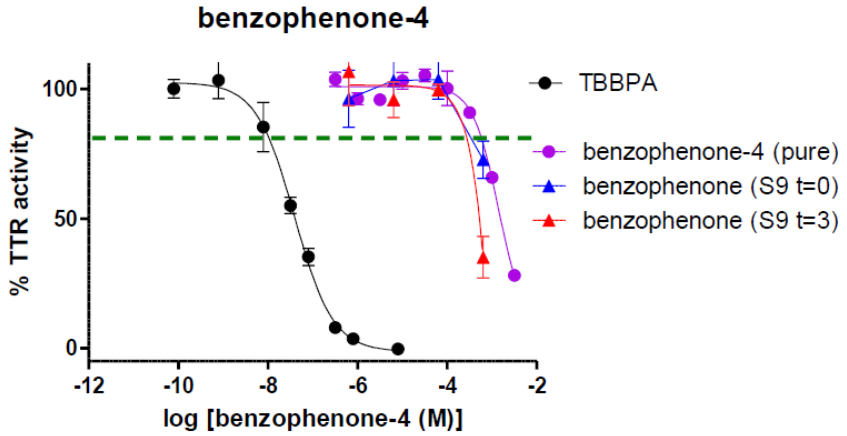
Renal biomarkers (PTC)

- No significant response for BP-4
- Positive controls (Cisplatin and Omeprazole gave expected dose-response at 72-h)

hTPO inhibition assay results



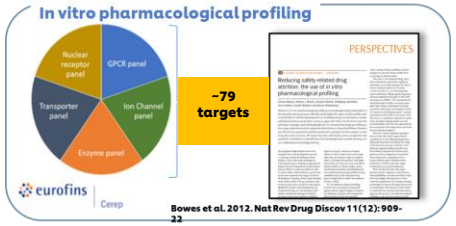
TTR-TRβ assay results



Module 3- Risk characterisation

BIOACTIVITY

In vitro pharmacological profiling



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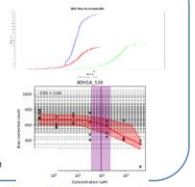

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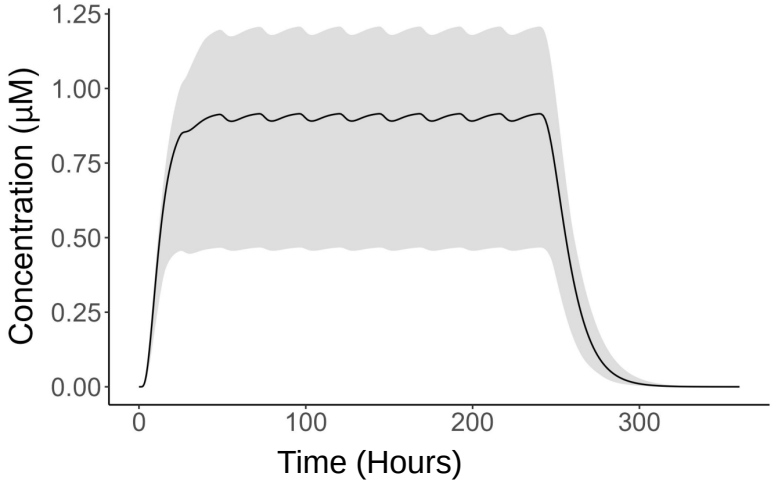
- KIM-1, NGAL, Clusterin,
- TEER (Day 0 and Day 3), ATP, LDH
- Toxicogenomics (3 donors, 2 duplicates per donor), 8 concentrations, 24h and 72h timepoints
- Omeprazole and cisplatin added as benchmarks/positive controls

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

EXPOSURE



Identify lowest (most sensitive) point of departure, expressed in µM

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

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

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

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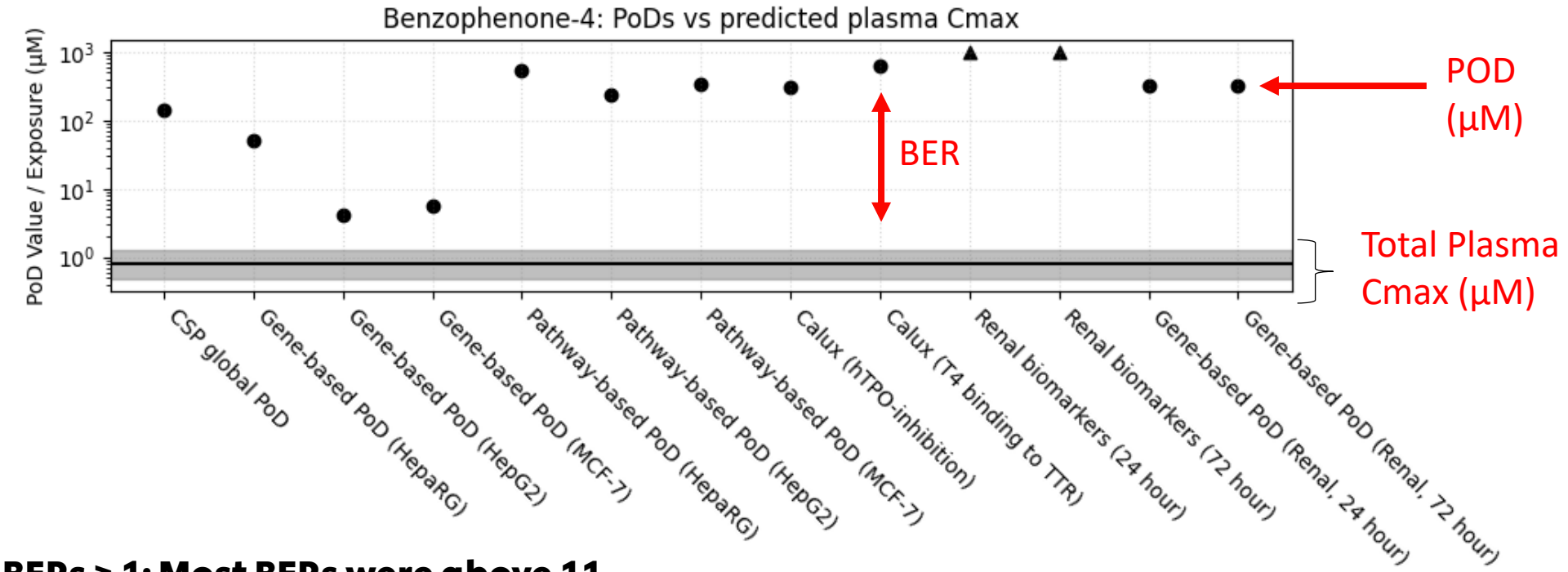
Bioactivity: exposure ratio calculation: BER ranging from 3.3-496



- **All BERs > 1; Most BERs were above 11**
- **Lowest BER (3.4):** PODs was obtained from HTTr in HepG2 cells when the BIFROST method was used (POD of 4.2 µM). BER obtained from pathway level POD was 189.
- **Highest BER (496):** PODNAM derived from the Calux assay (T4 binding to TTR).

Conclusions & reflections

NAM-based assessment for 5% inclusion of BP-4

Lowest BER= 3.3

BER range= 3.3-496

Conclusion

Low risk considering weight of evidence and model/PoD relevance

Traditional animal assessment for 5% inclusion of BP-4

NOAEL= 1239 mg/kg bw/day

Adjusted for oral absorption= 620 mg/kg bw/day

Exposure= 0.069 mg/kg bw/d

Margin of Safety (MoS)= 8986

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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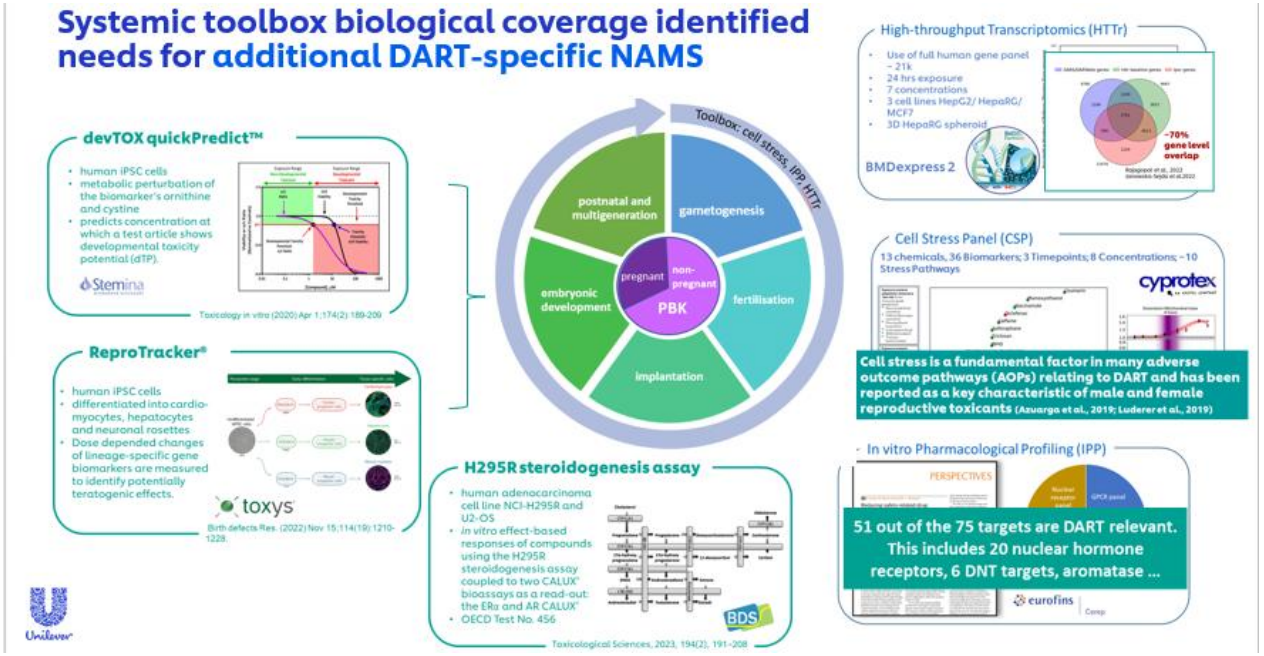
Other research areas: DART & Complex in vitro models



Muller et al., accepted for publication

Establishing human liver microphysiological coculture system for higher throughput chemical safety assessment

BROWN

Aim: to develop 2-chamber liver-organ coculture model in a higher-throughput 96-well format for the determination of toxicity on target tissues in the presence of human liver biology and metabolism.

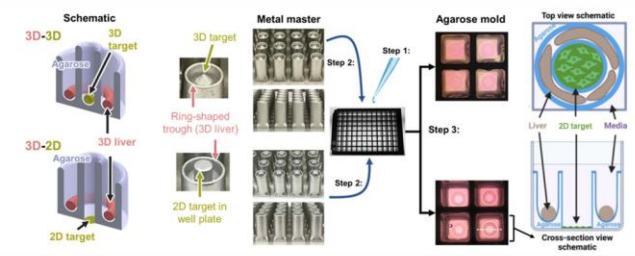
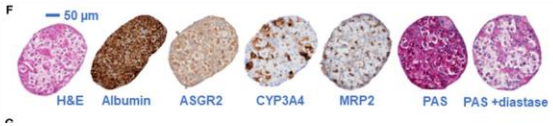


Figure. Schematic of 3D co-culture in agarose gel moulds, showing 3D toroid of HepaRG cells on the outer ring and 2D AR-CALUX cells as a target for metabolites in the centre of the mould.

Key characteristics of the system:

- Culture medium and compounds freely diffuse between the 2 chambers
- 3D HepaRG function and phenotype:
  - Robust protein expression of liver biomarkers (albumin, asialoglycoprotein receptor, Phase I cytochrome P450 [CYP3A4] enzyme, MPR2, and glycogen), and exhibited Phase I/II enzyme activities over the course of 17 days



Ip et al.,2024. https://doi.org/10.1093/toxsci/kfae018;

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Conclusions & reflections

- Case studies have demonstrated it is possible to integrate exposure estimates and bioactivity points of departure to make a safety decision.
- These case studies showed that the approach 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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