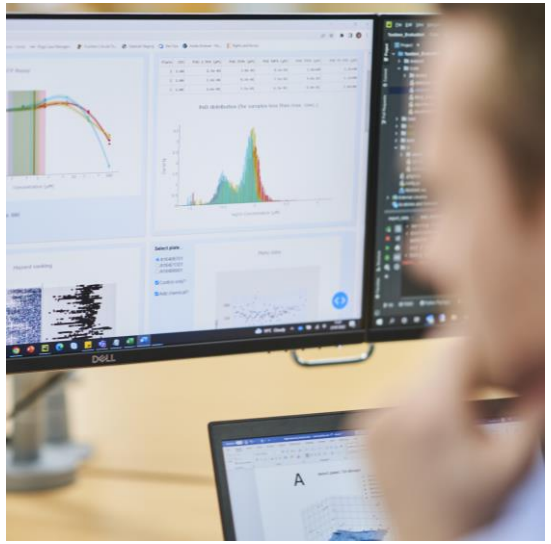
Next Generation Risk Assessment (NGRA) using New Approach Methods (NAMs) to Evaluate Systemic Safety for Consumers Using Benzophenone-4 as a UV-filter in a Sunscreen Product

Maria Baltazar, Unilever Safety and Environmental Assurance Centre, UK

COSMETICS EUROPE LRSS



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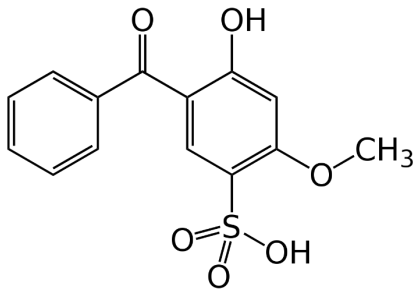
Benzophenone-4 (BP-4) case study: Objectives & Approach



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**
- 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%?

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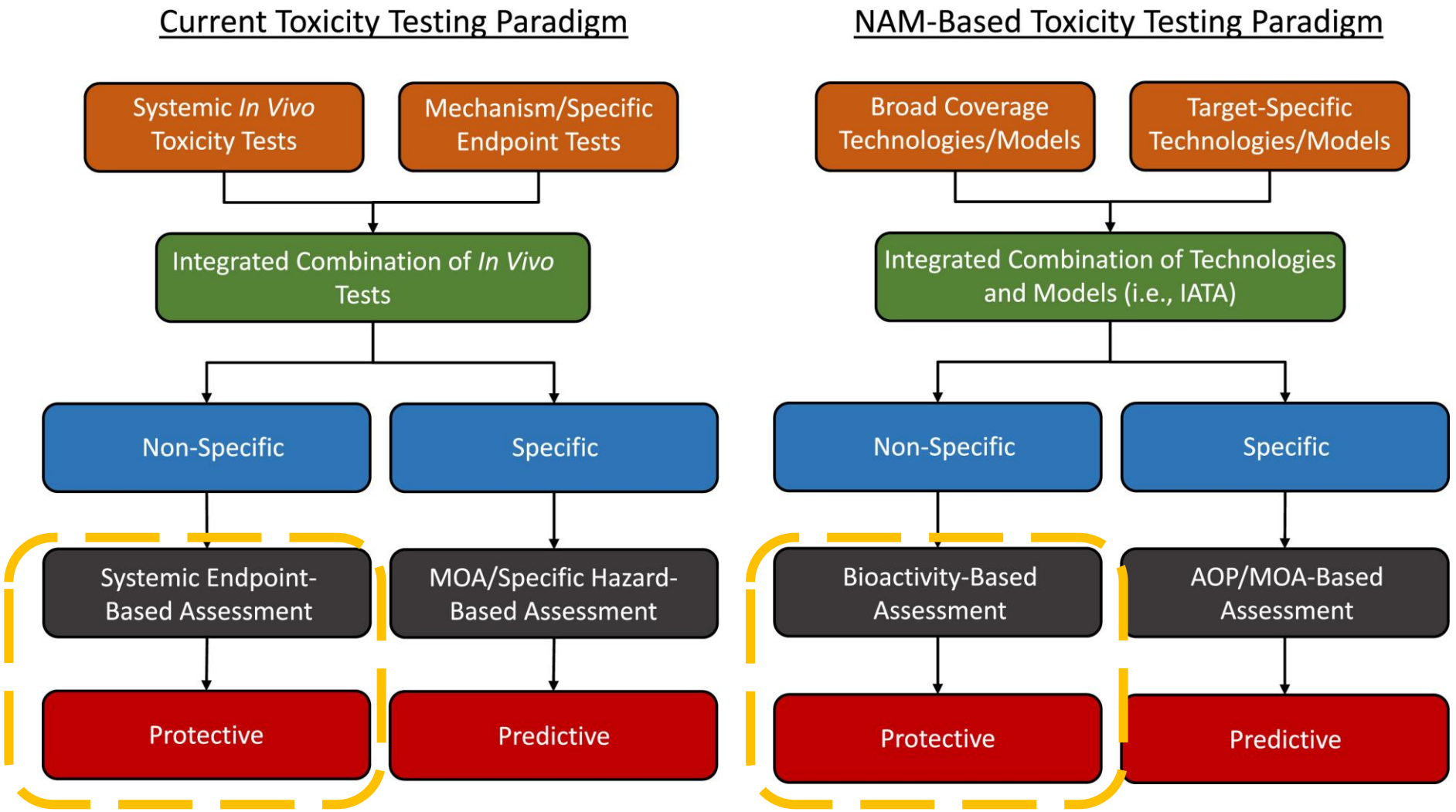
Context of use: bioactivity based-assessment and protection of human health



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

Overall approach for Benzophenone-4 (BP-4)

OS02: Short Orals Tuesday, 10h00 – 12h00- Ans Punt
"Establishing scientific confidence in PBK models for QIVIVE in the absence of in vivo kinetic data"

Identified use scenario

Identified molecular structure

Collected existing data → TTC not possible
Assumed no animal or human data available

Module 1- Exposure estimation

Generated in vitro ADME data and Performed PBK modelling to derive plasma C_{max} estimation) → See Punt et al., 2024

Generic hypothesis: Biological activity measured using a broad suite of human-relevant test systems is sufficiently protective. If bioactivity is not observed at concentrations experienced systemically in consumers, then there are no adverse effects. PBK model indicated that concentrations of BP-4 are limited to liver and kidney, therefore a relevant kidney cell model was included in the testing strategy in addition to the already liver cell line. In silico tools predicted binding to estrogen receptor.

Module 2- Bioactivity characterisation

Systemic NAM-toolbox[1,2]

Broad suite of assays and analysis used as part of the systemic toolbox as outlined in Middleton et al:

- Cell stress panel (CSP) in HepG2 cells
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EATS activity: estrogenic, androgenic, thyroidogenic and steroidogenesis using CALUX assays

Investigated BP-4 toxicity in the primary human proximal tubule model (aProximate™)

Tools to address specific risk assessment questions

Module 3- Risk characterisation

Calculation of Bioactivity-Exposure ratio (BER). Assessment based on lowest of POD_{NAM} together with weight of evidence

Risk evaluation and risk assessment documentation

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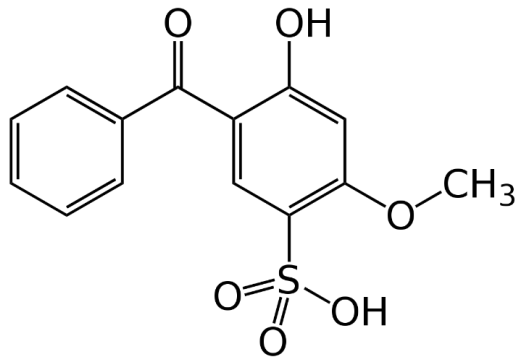
Gathering information: Alerts from *in silico* tools



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

- **Results:**

  - **Benzophenone-4 did not trigger many alerts within the tools used**. The most common alert across the tools was for skin sensitisation, or protein binding as an indication of skin sensitisation, in the DEREK, TIMES and OECD Toolbox outputs.

  - No alerts for DNA binding, no systemic alerts including DART alerts, no androgen agonism/antagonism

  - Very few predicted metabolites (via hydroxylation and demethylation)

  - **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.

Skin sens out of scope

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Module 1: steps to estimate internal exposure

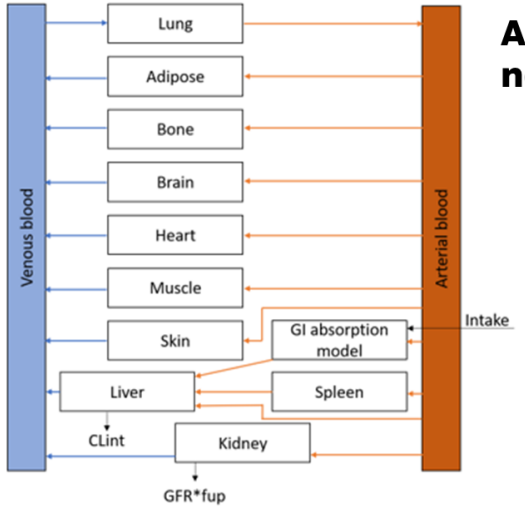
Exposure scenario (applied dose)

- 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

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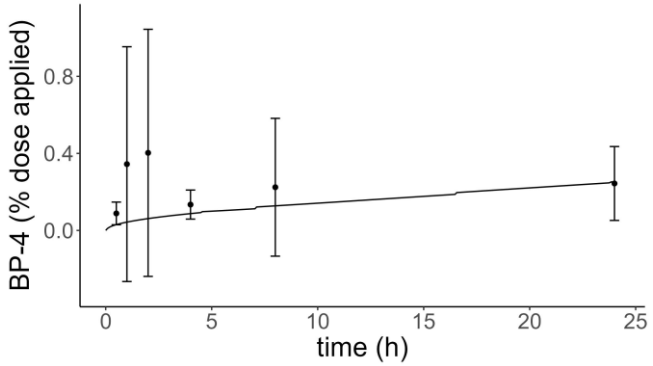
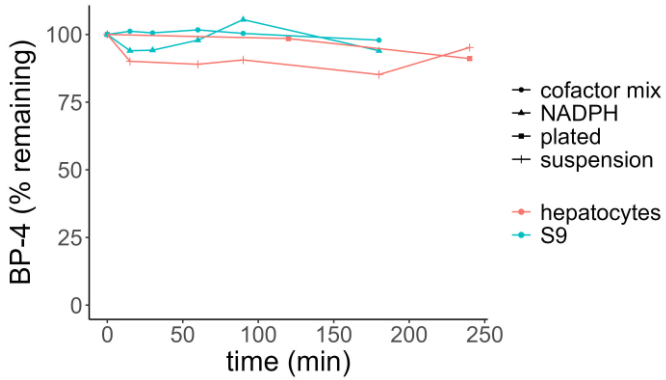
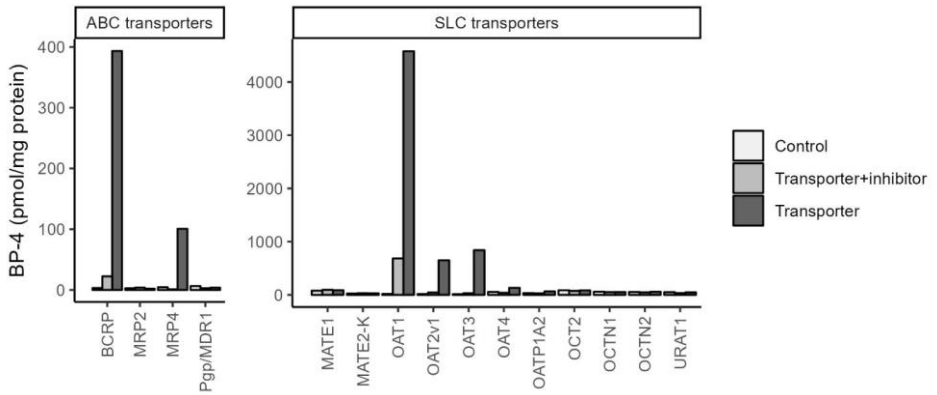
Module 1: Key ADME findings

- 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

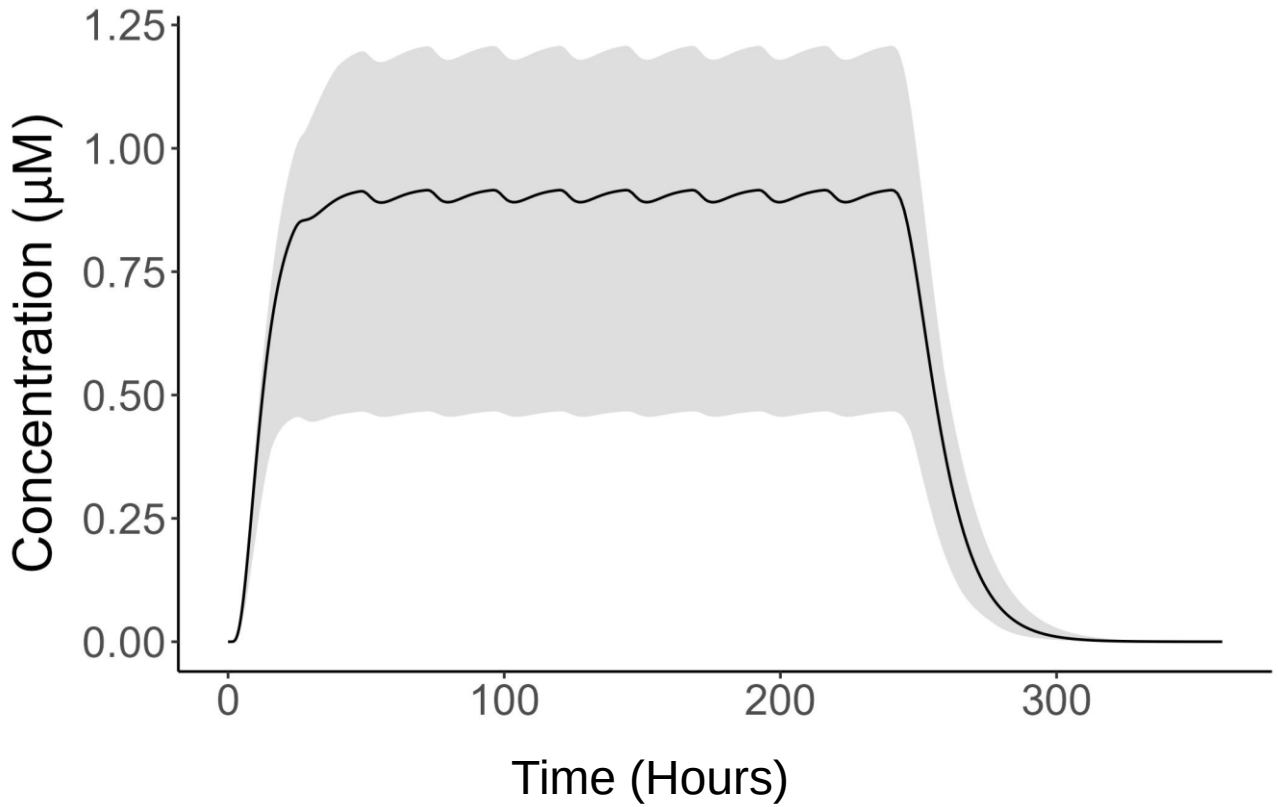


Figure. Population PBK simulation results (time course data and C_{max}) on benzophenone-4 concentrations in plasma after repeated exposure of body lotion 18g/day, i.e., 9g two times per day for a period of 10 days, with 5% benzophenone-4, on the whole body.

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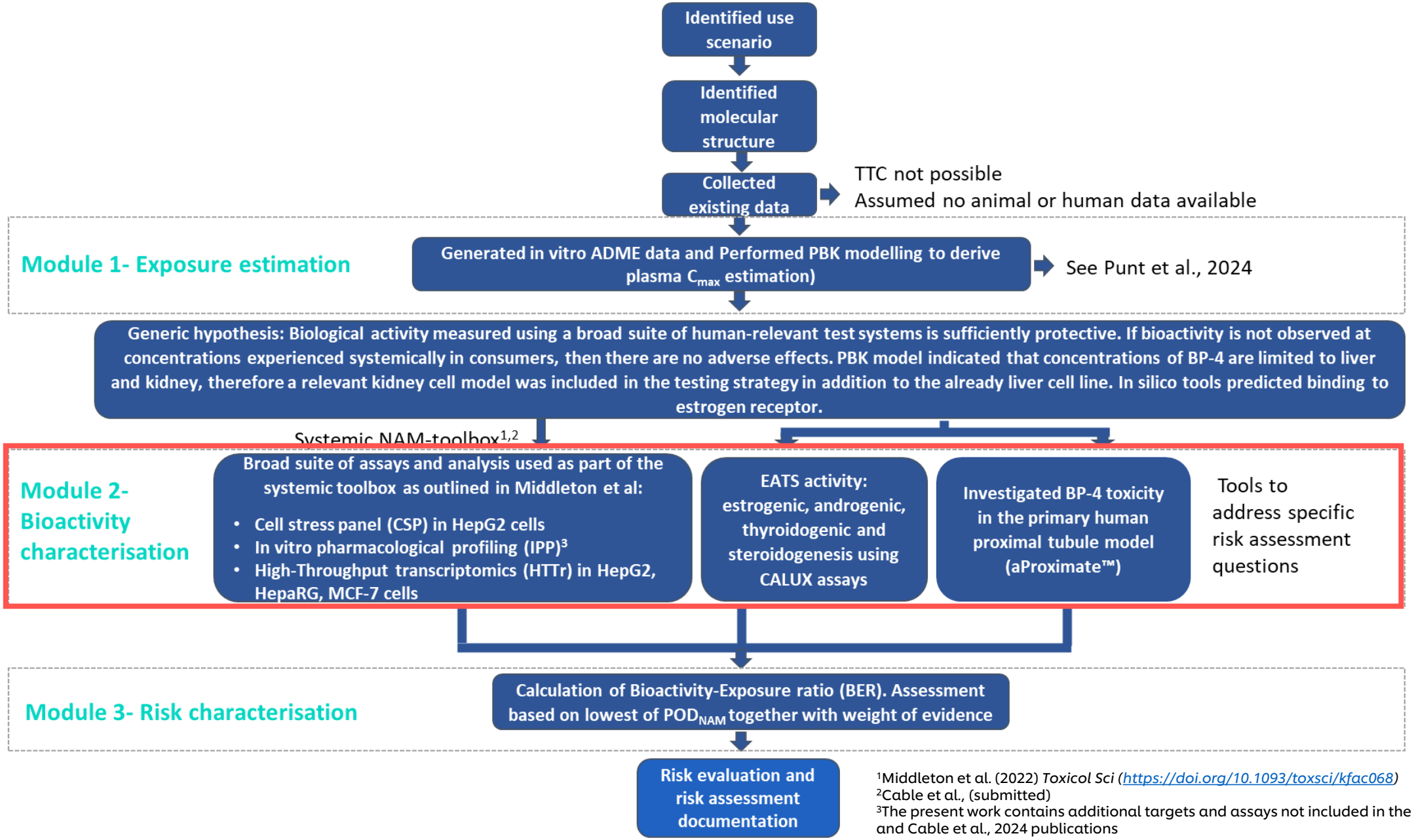
Problem formulation after collating existing information and exposure estimation

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	• 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)

Overall approach for Benzophenone-4 (BP-4)



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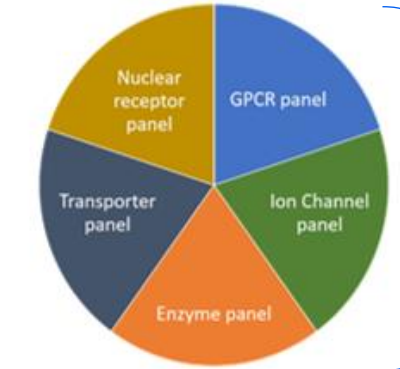
Module 2: Broad suite of assays and analysis used as part of the systemic toolbox

Transcriptomics was applied as a broad nontargeted biological screen

In vitro pharmacological profiling



~79 targets

eurofins | Cerep


PERSPECTIVES

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

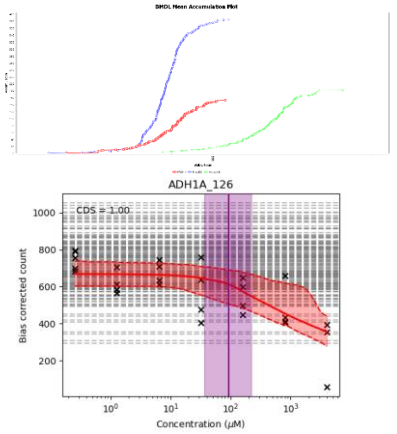
Joanne Bowes, Andrew J. Brown, Jacques Hamon, Wolfgang Jarolimek, Arun Sridhar, Gareth Waldron and Steven Whitebread

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

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

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

Cell stress panel (CSP)

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

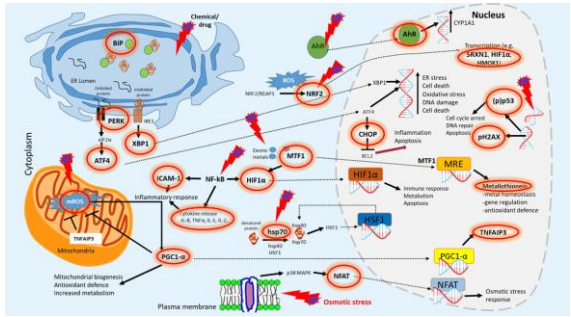


Image kindly provided by Paul Walker (Cyprotex)

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

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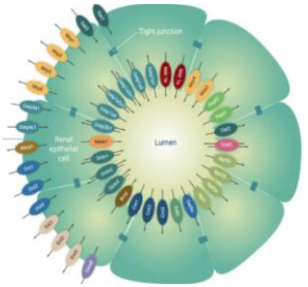
Module 2: Tools to address specific risk assessment questions

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

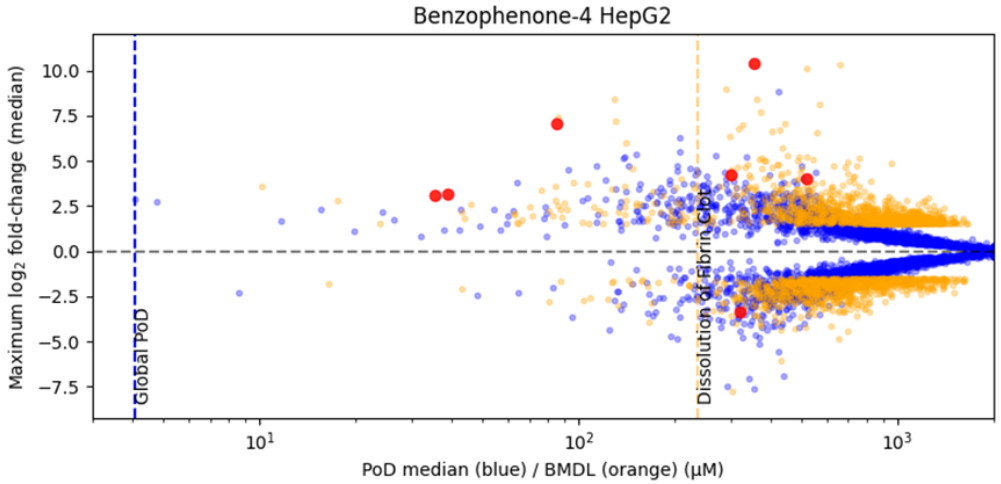
Cell Stress Panel

- Global POD_{NAM} = 140 µM

In vitro Pharmacological profiling

- Tested up to 100 µM
- ~83 targets compiled by Cosmetics Europe Safety pharmacology WG
- **2 hits** (PXR and PDE4D) (IC50 of 36 and 92 µM, respectively)

HTTr



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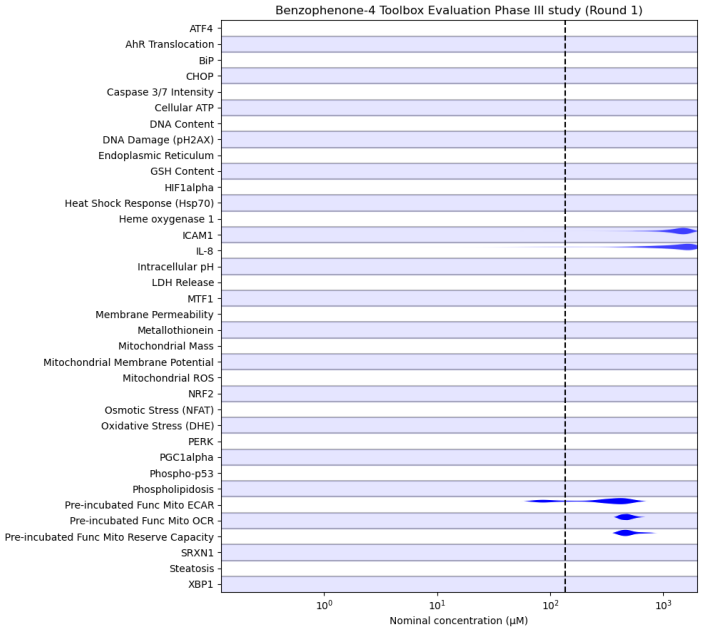


Key Results & Deriving Points of Departure (PoDs)

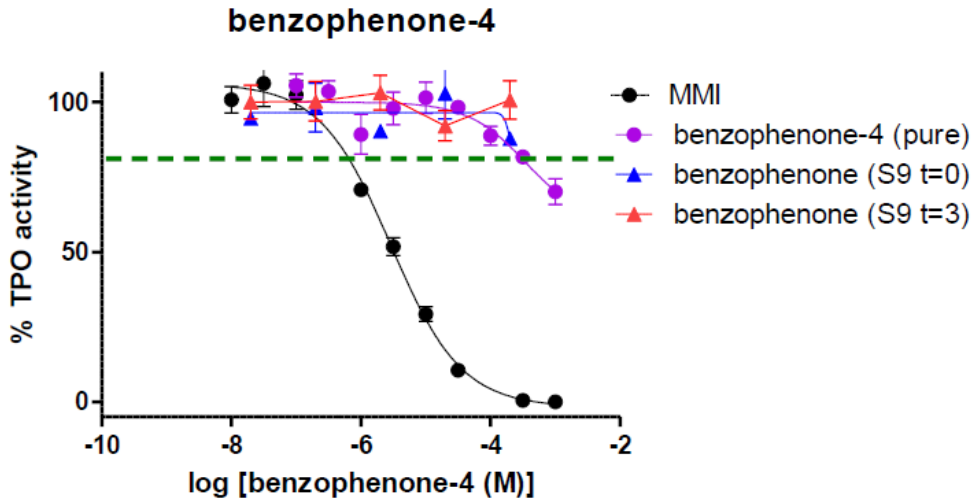
Calux assays

- 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).

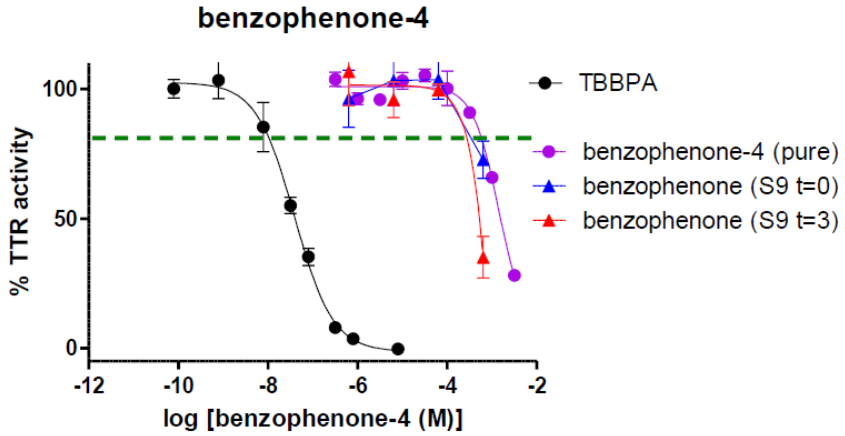
Renal biomarkers (PTC)

- 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



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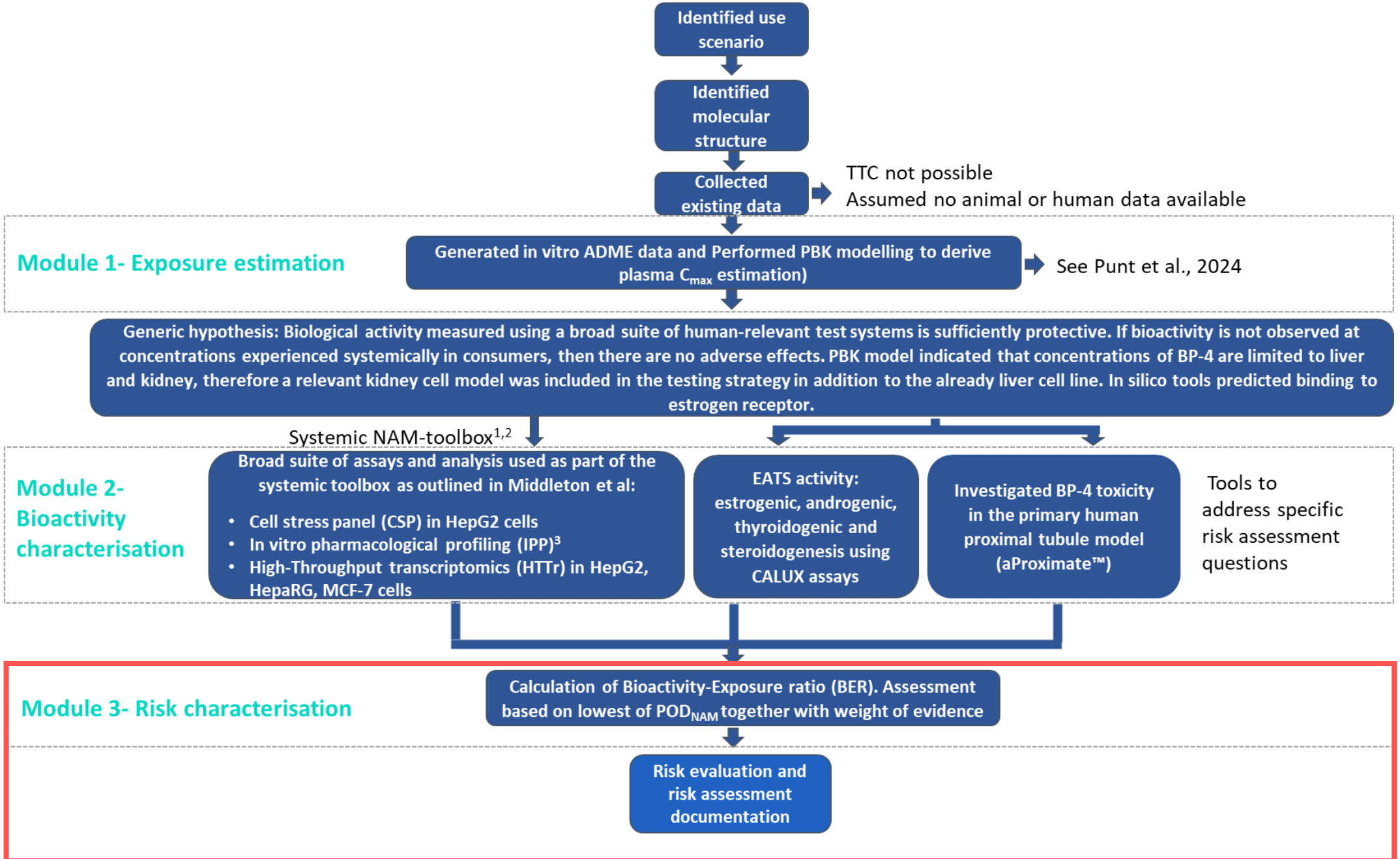
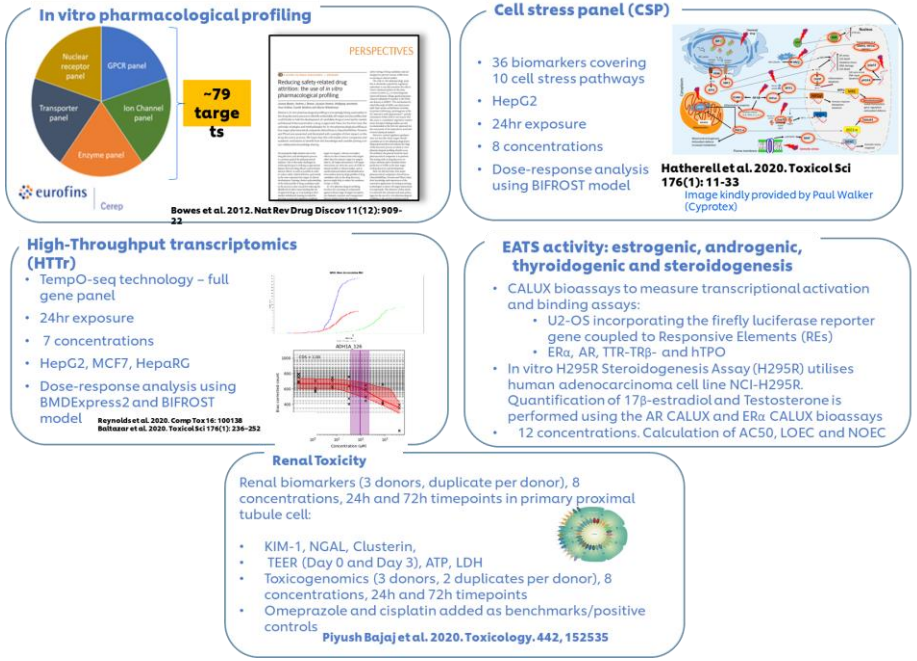
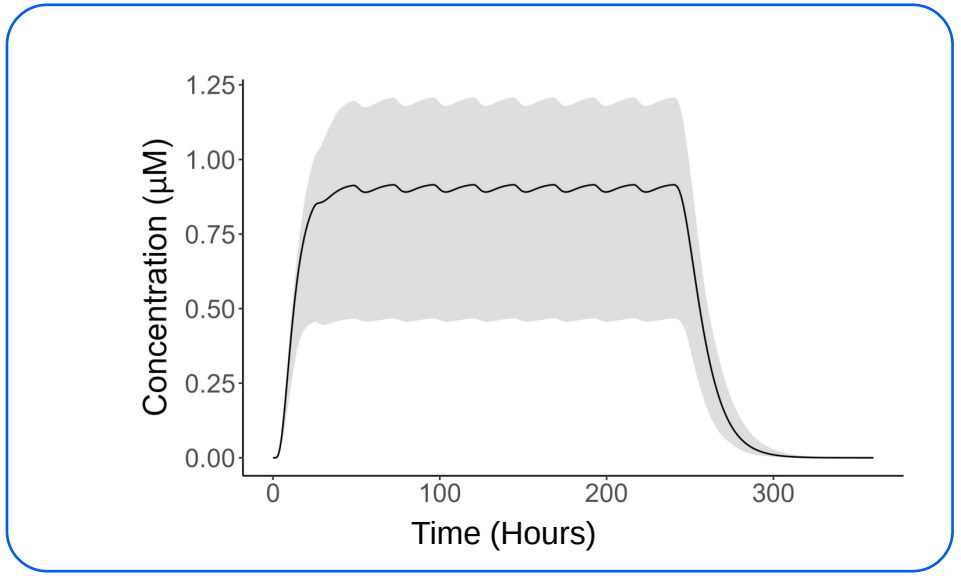


Module 3- Risk characterisation

BIOACTIVITY



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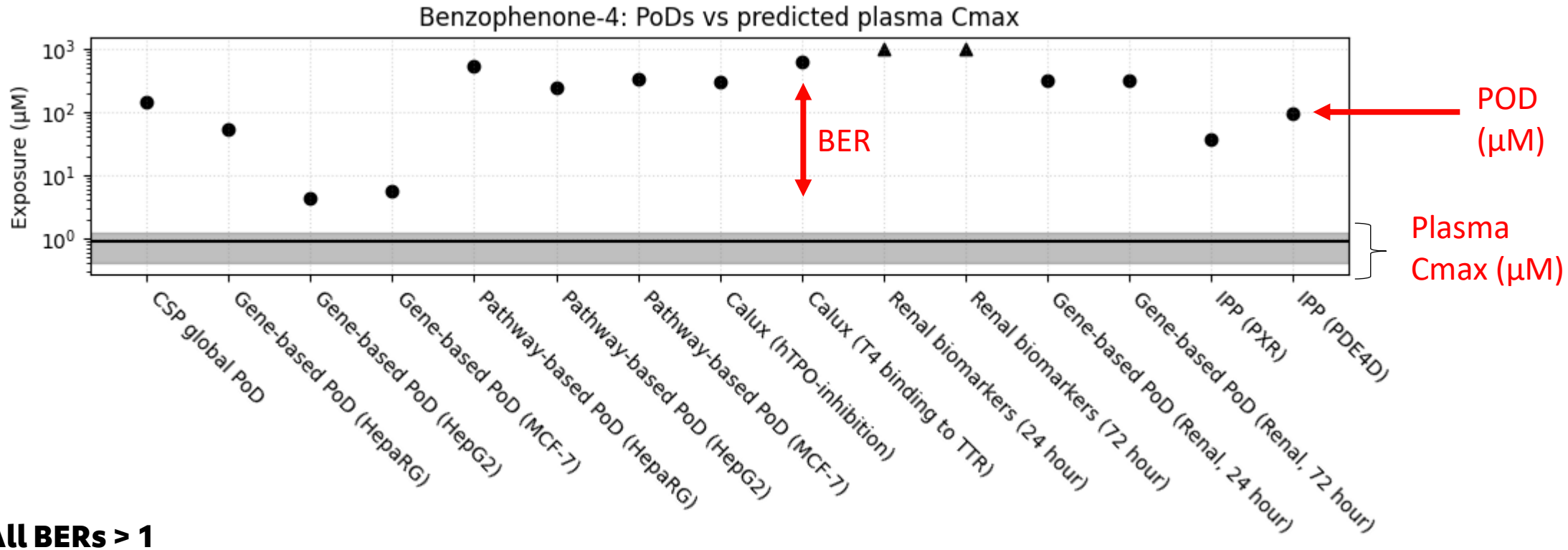
Bioactivity: exposure ratio calculation: BER ranging from 3.4-508



- **All BERs > 1**
- **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 193.
- **Highest BER (508):** PODNAM derived from the Calux assay (T4 binding to TTR).

When is a BER sufficiently protective?

Conceptually, with the following assumptions a BER>1 indicates a low risk of adverse effects in consumers following use of the product:

a) The in vitro measures of bioactivity provide appropriate biological coverage

b) There is confidence that the test systems are at least as sensitive to perturbation as human cells in vivo

c) The exposure estimate is conservative for the exposed population

Safety assessment discussion

- Lowest BER across all PODs was obtained from HTTr in HepG2 cells when the BIFROST method was used (POD of 4.2 µM)
  - *Single gene change of CYP 1A1*
  - *Lowest BMDL in the same cell line is 240* µM (BER of 193)
  - This provides some assurance that the gene changes seen at 4.1 µM may be of limited toxicological significance.
- The high IC50s and LOEC obtained in the pharmacology and endocrine panels combined with the unique kinetic profile (i.e. limited distribution) indicate that BP-4 is unlikely to exhibit specific modes of toxicity not covered by the NAMs tested.

Conclusion: *Based on the tools and test systems used in this assessment and the assumptions used in the risk assessment, internal exposures would need to be greater than those predicted to lead to toxicologically significant systemic biological activity in consumers.*

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Conclusions & reflections

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

Lowest BER= 3.4

BER range= 3.4-508

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

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Andrew White
Richard Cubberley
Sandrine Spriggs
Ruth Pendlington
Katie Przybylak
Alistair Middleton

BP4 Consortium
Cosmetics Europe/LRSS Case study Leaders Team
Pharmacelsus
Eurofins
BioClavis
Cyprotex
SOLVO
BioDetection Systems
NewCells

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