Advancing the Application of New Approach Methodologies (NAMs) for Systemic Toxicity Assessment of cosmetic ingredients: an example with UV filters

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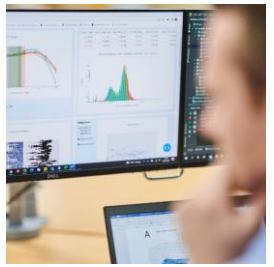
COSMETICS EUROPE LRSS

SERS
Safety, Environmental & Regulatory Science



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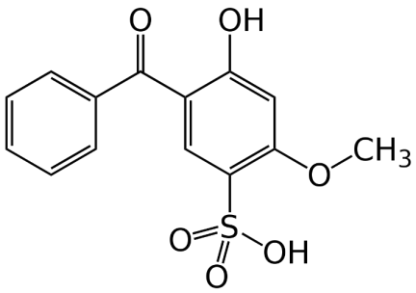
Benzophenone-4 (BP-4) case study: Objectives & Approach



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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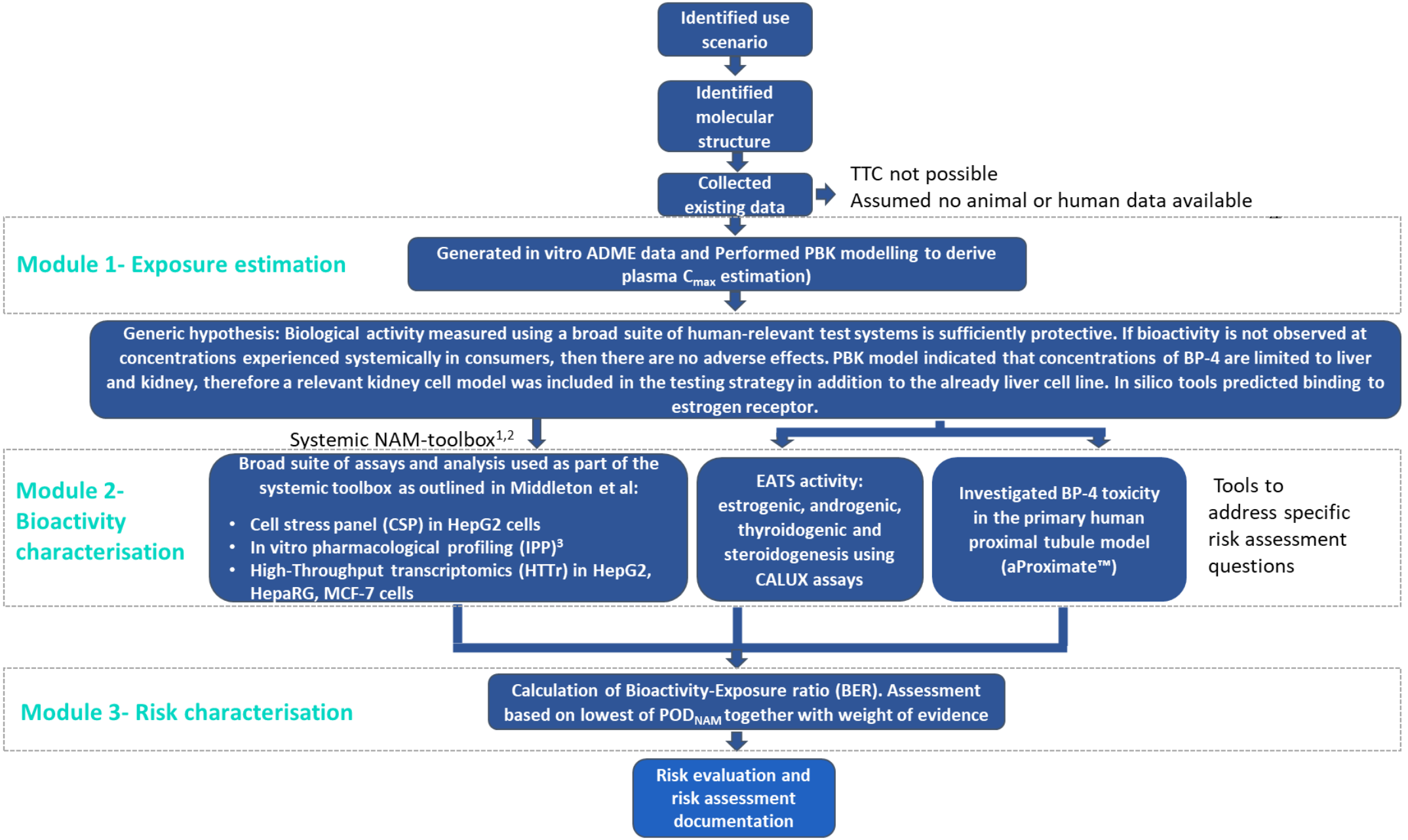
Overall approach for Benzophenone-4 (BP-4)



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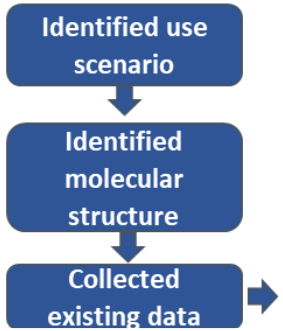
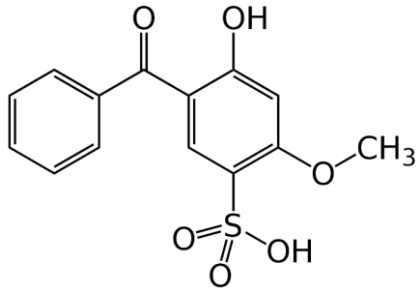


TTC not possible
Assumed no animal or human data available



Tool	Output
DEREK Nexus	Alerts for skin sensitization, photoallergenicity, and carcinogenicity.
METEOR Nexus	Very few predicted metabolites for BP-4
OECD QSAR Toolbox	BP-4 was predicted to have a low order of toxicity, with none of the models predicting DNA binding. Mixed alerts for protein binding. No alerts with respect to developmental and reproductive toxicity.
OPERA	No alerts for androgen receptor or estrogen receptor agonism or antagonism.
VEGA	No alerts for androgen receptor binding; possible estrogen receptor activity; predicted non-developmental and reproductive toxicant.

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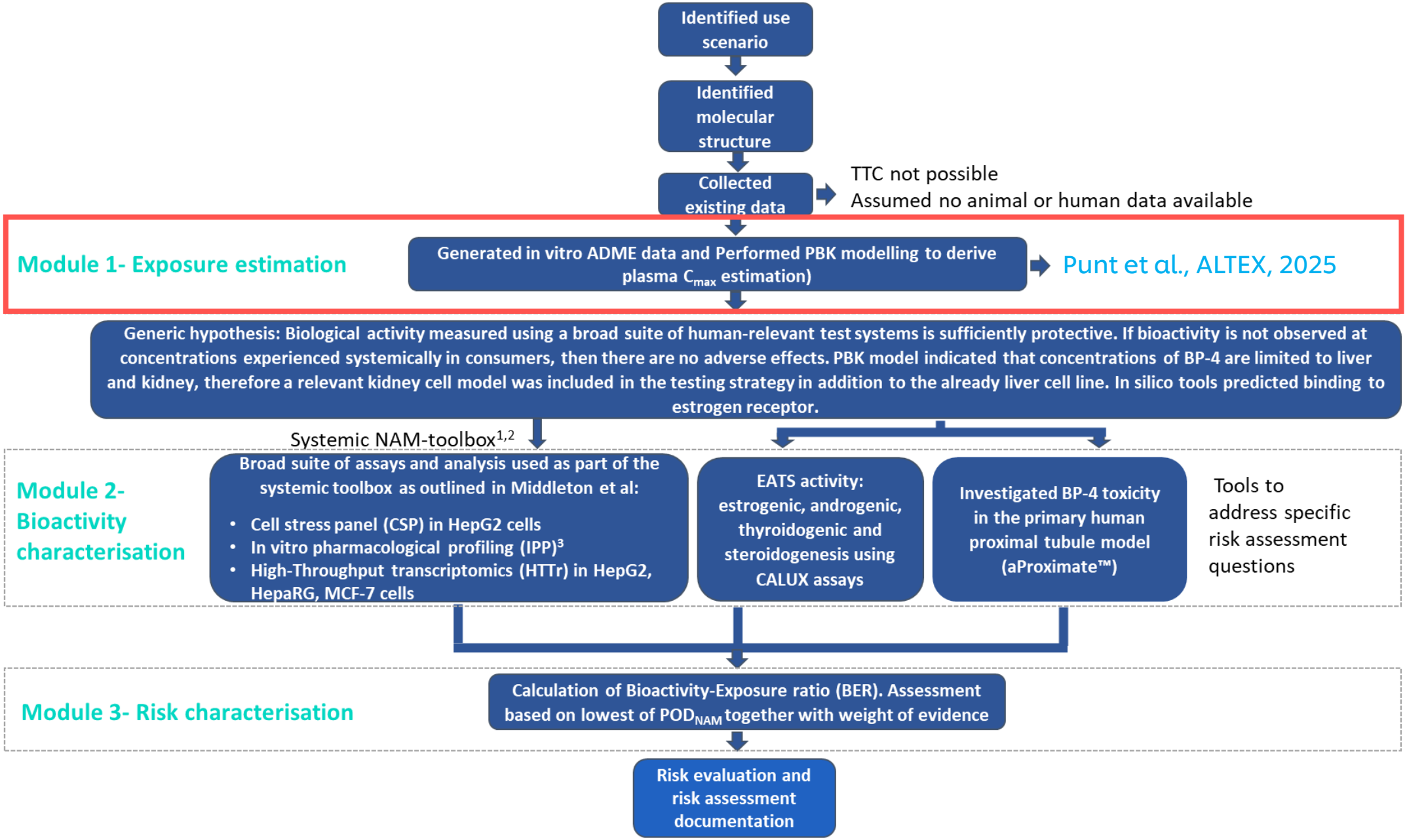


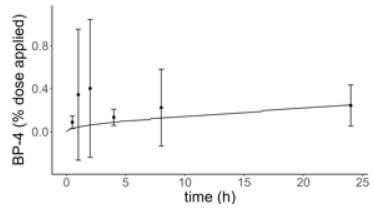
Module 1: steps to estimate internal exposure

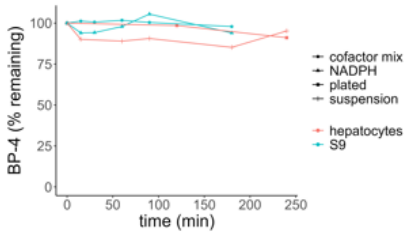
Exposure scenario (applied dose)

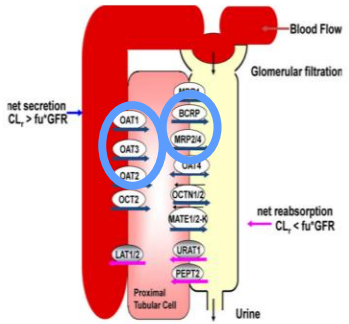
- 5% in Sunscreen product,
- 18g/day, two times, 9g/application (as per SCCS notes of guidance)
- On body and face 17500cm2 (total body area)

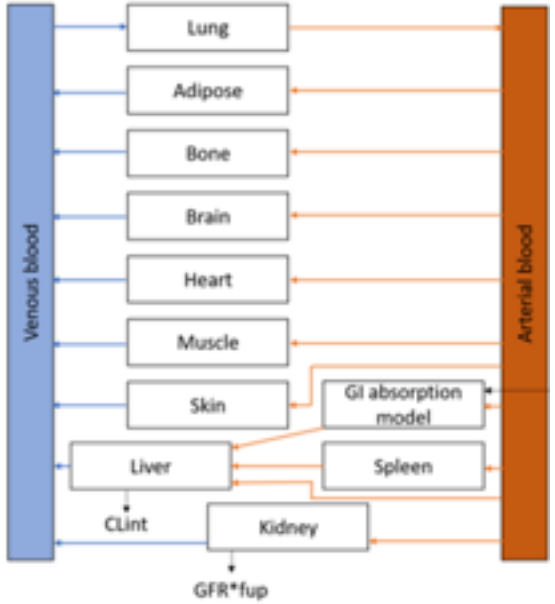
ADME data for model building

- 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)









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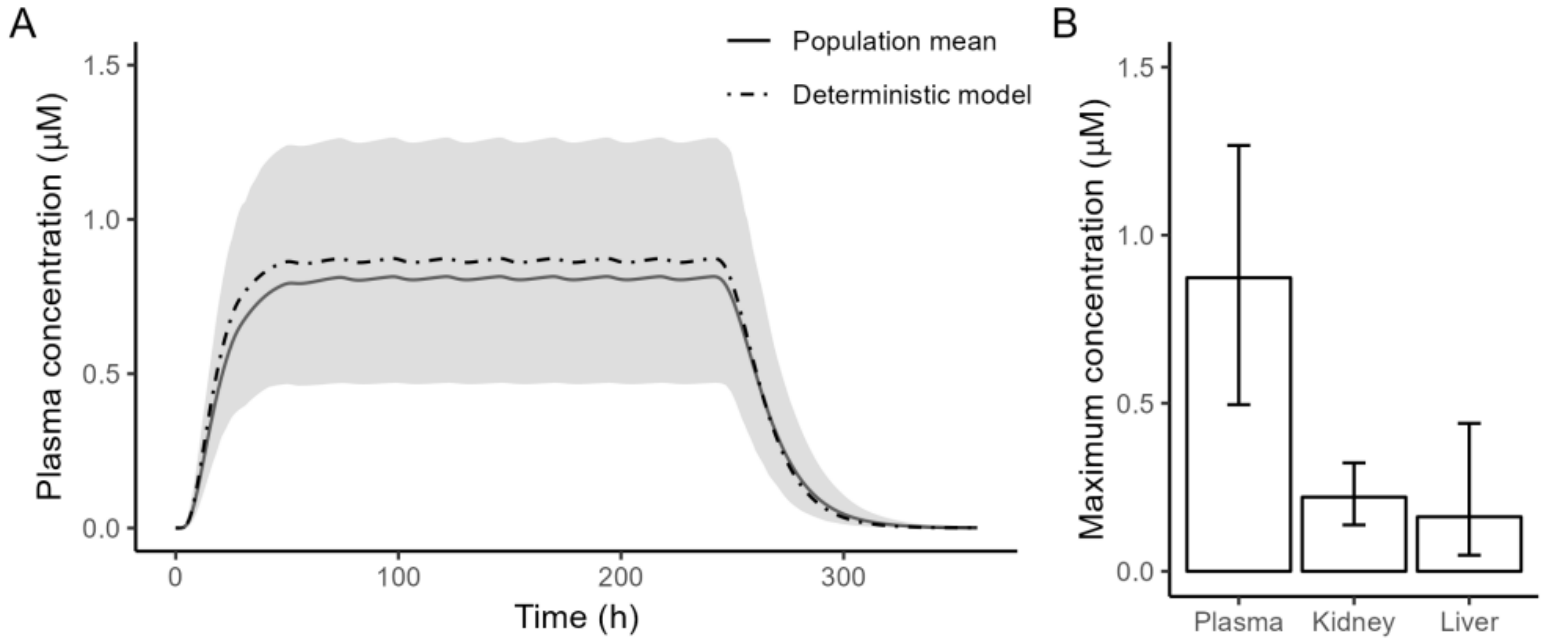
Module 1: plasma Cmax prediction for the population

- **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



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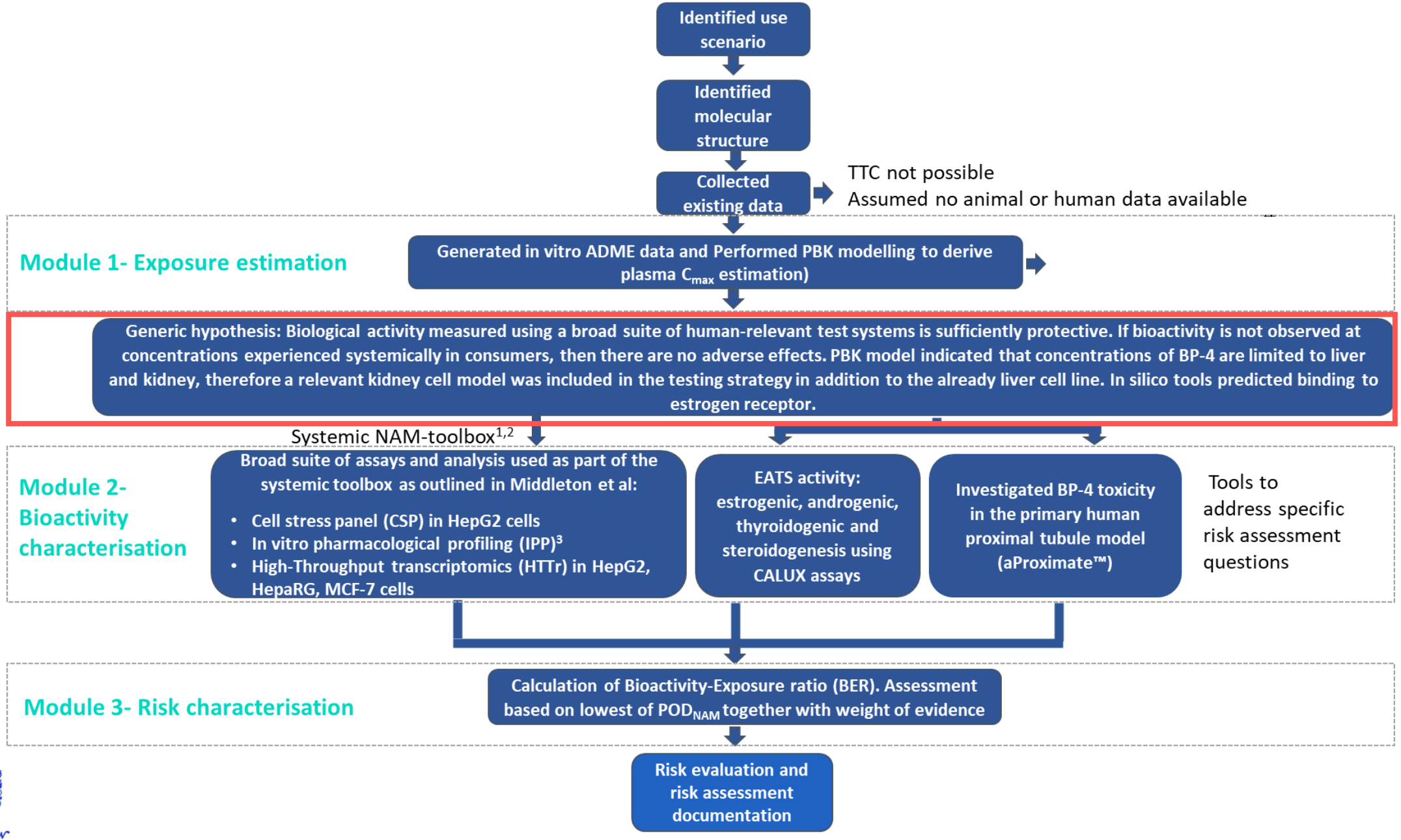

Problem formulation after collating existing information and exposure estimation

Hypothesis	Testing strategy
• Absence of in silico alerts ≠ no toxicity	• **Test the Systemic NAM Toolbox** using non targeted (transcriptomics in HepG2, HepaRG, MCF7, cell stress panel) & targeted NAMs (in vitro pharmacological profiling)
• 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.
• 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

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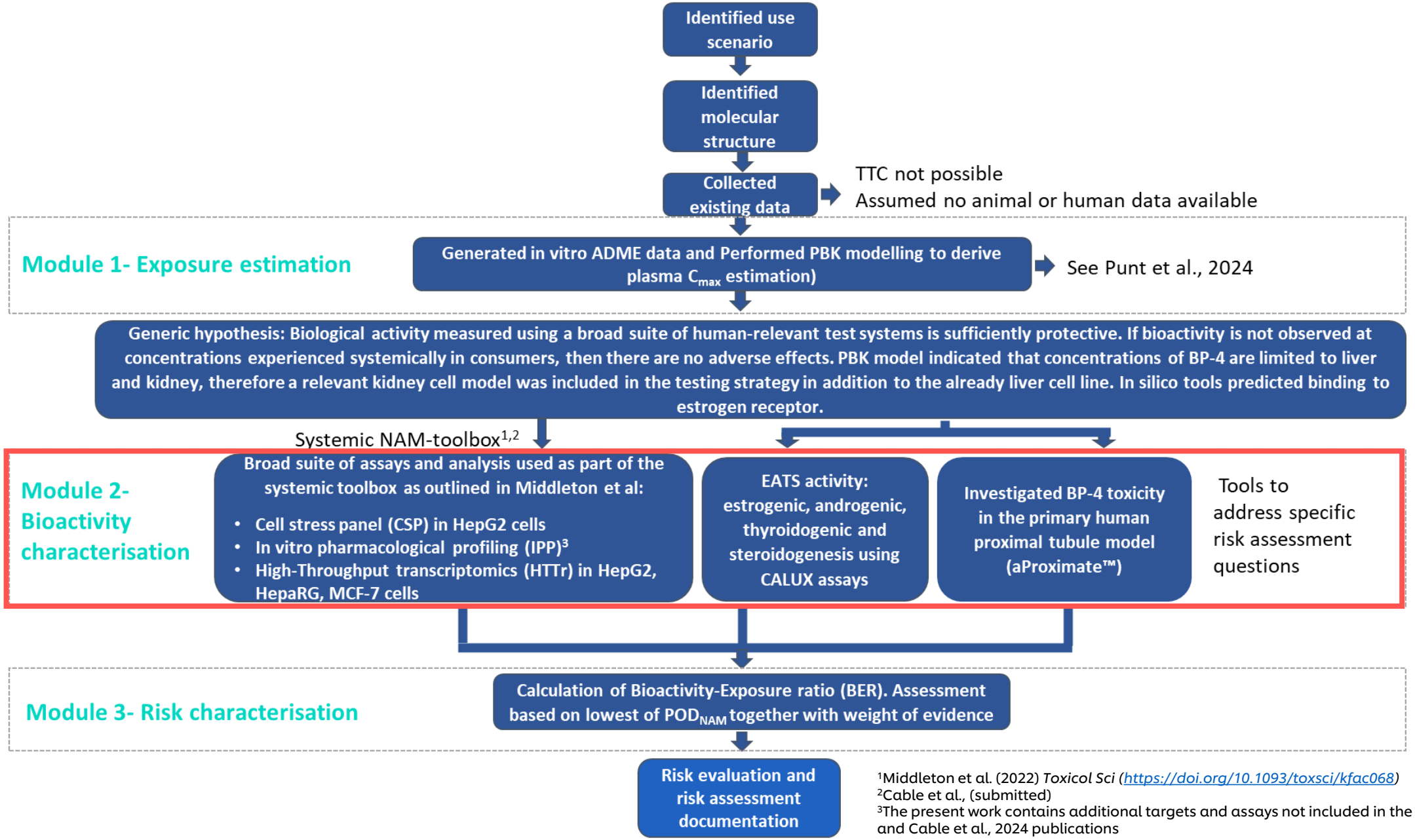
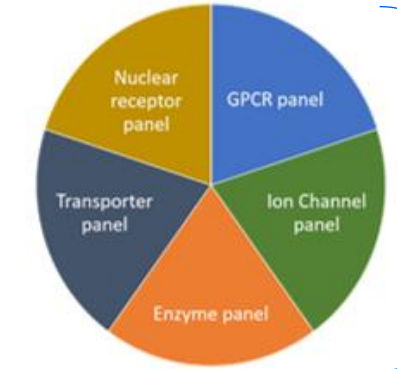


[1]Middleton et al. (2022) *Toxicol Sci* (https://doi.org/10.1093/toxsci/kfac068)
[2]Cable et al., (submitted)
[3]The present work contains additional targets and assays not included in the Middleton et al., 2022 and Cable et al., 2024 publications

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

Transcriptomics was applied as a broad non-targeted biological screen

In vitro pharmacological profiling



~79 targets


PERSPECTIVES

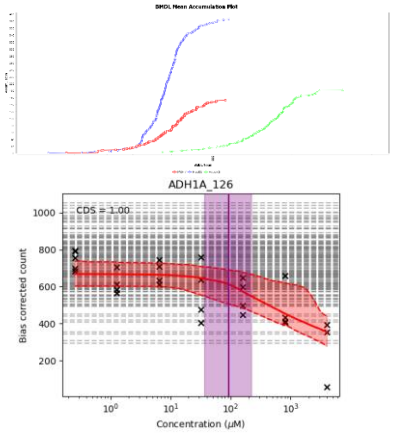
Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

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

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

Cell stress panel (CSP)

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

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

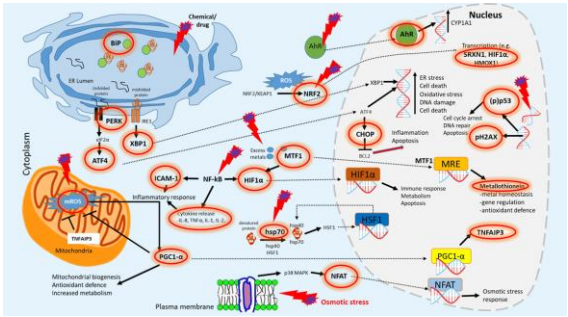


Image kindly provided by Paul Walker (Cyprotex)

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

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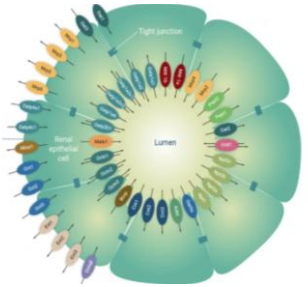
Module 2: Tools to address specific risk assessment questions

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

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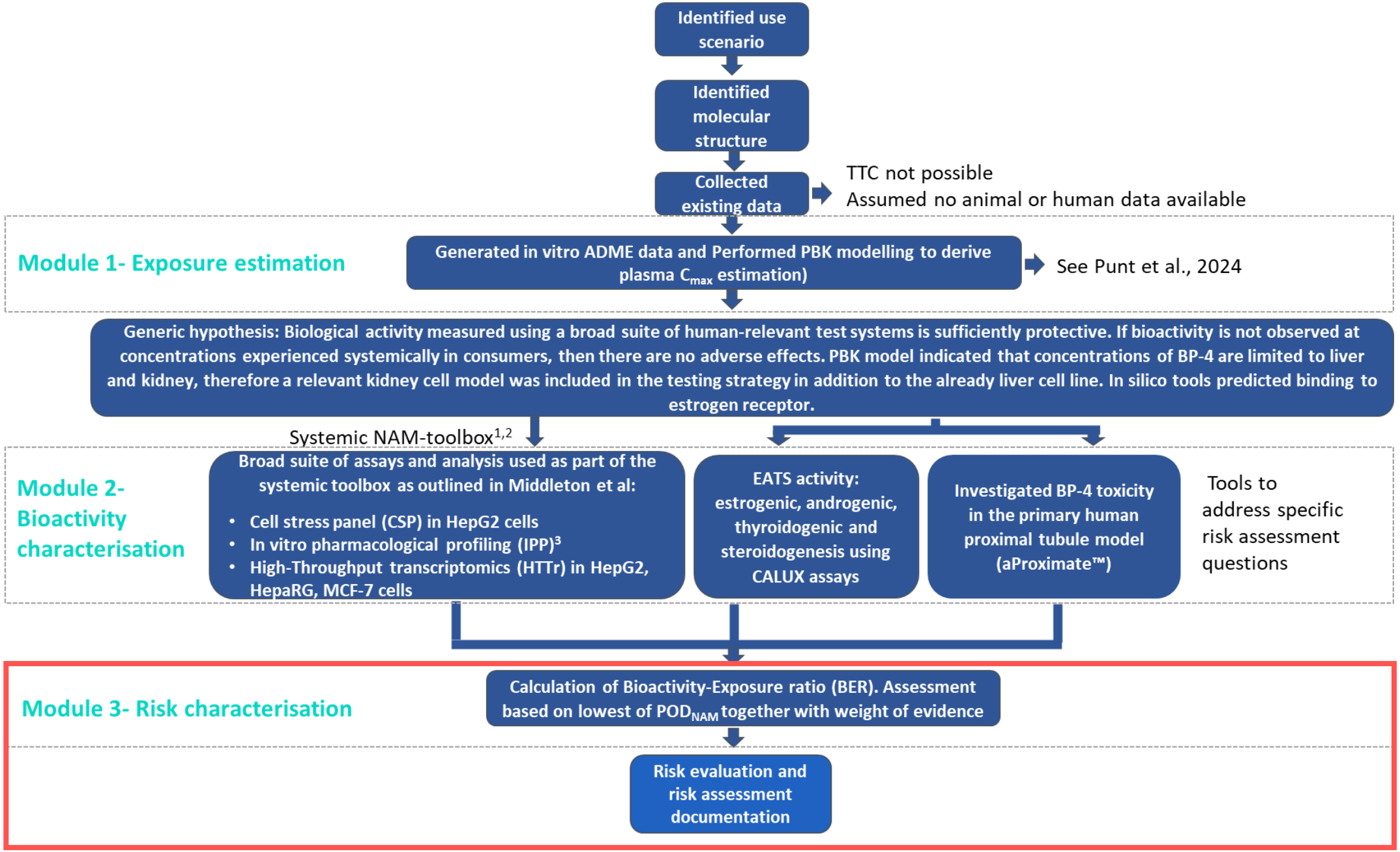

Module 3- Risk characterisation

BIOACTIVITY

In vitro pharmacological profiling

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

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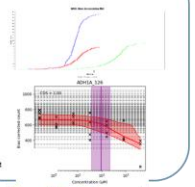

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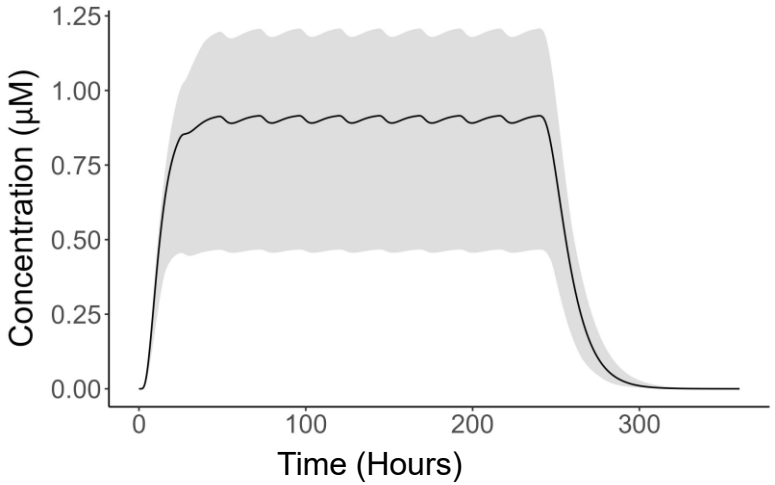
EXPOSURE



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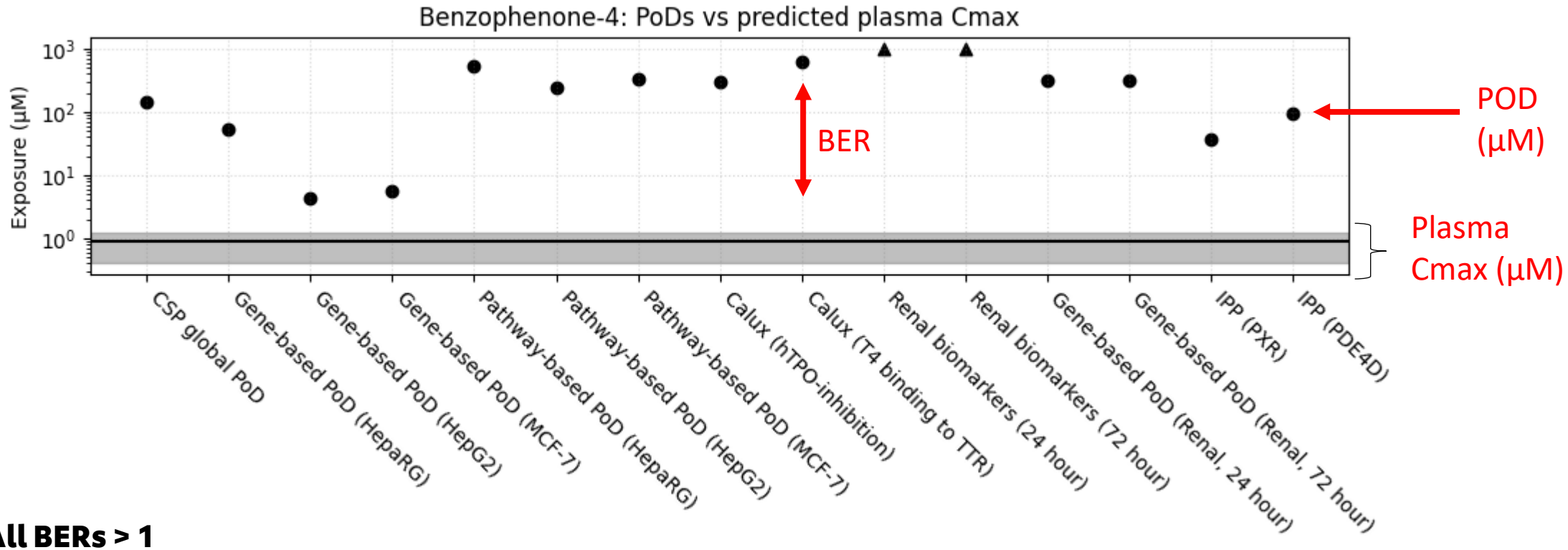
Bioactivity: exposure ratio calculation: BER ranging from 3.4-508



- **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

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

- Cable et al (2025) Tox Sci (*https://doi.org/10.1093/toxsci/kfae159*)
- 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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Pharmacelsus
Eurofins
BioClavis
Cyprotex
SOLVO
BioDetection Systems
NewCells

ALTEX. 2025;42(3):511-530. doi: 10.14573/altex.2501201. Epub 2025 May 19.

Making safety decisions for a sunscreen active ingredient using next-generation risk assessment: Benzophenone-4 case study

Maria T Baltazar [1], Sophie Cable [1], Richard Cubberley [1], Nicola J Hewitt [2], Jade Houghton [1], Predrag Kukic [1], Hequn Li [1 3], Sophie Malcomber [1], Beate Nicol [1], Ruth Pendlington [1], Ans Punt [1], Joe Reynolds [1], Sharon Scott [1], Sandrine Spriggs [1], Matthew P Dent [1]

Affiliations + expand

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Thank You

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