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

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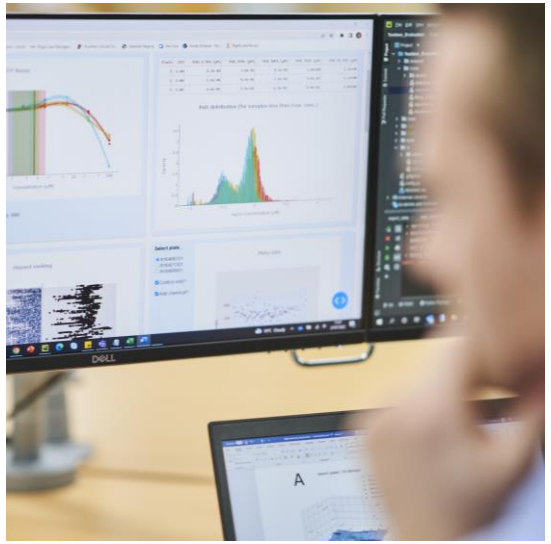

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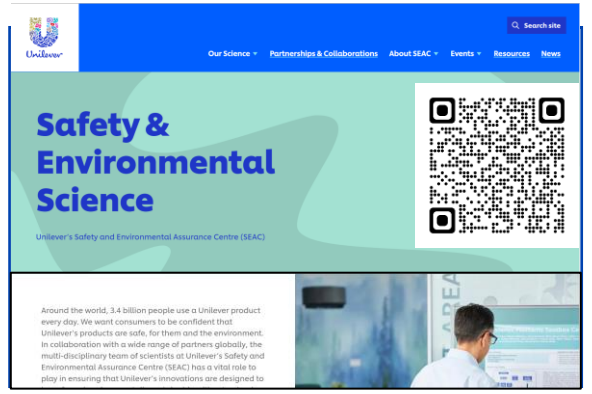
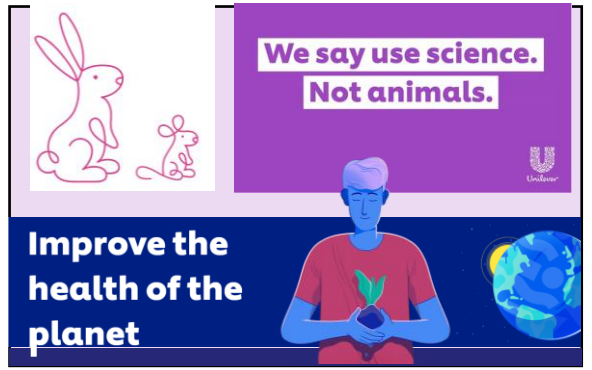
SEAC is Unilever's global centre of excellence in Safety & Sustainability Sciences, part of R&D's Safety, Environment & Regulatory Sciences Capability.

Diverse, multi-disciplinary team of ~150 scientists based at Colworth, UK; ~70 miles north of London

Highly collaborative, working with over 70 academic, industry, government & NGO partners worldwide





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Outline

- **Intro** – 5 mins
- **Problem formulation, in silico & exposure assessment** – 20 mins
- **Breakout discussion I**– 20 mins + 5 mins feedback
- **Bioactivity characterisation and risk assessment conclusion-** 15 mins
- **Breakout discussion II** – 20 mins + 5 mins feedback
- **Discussion** – 10 min

Purpose of the Workshop

- Make participants familiar with some of the available *in silico* and *in vitro* NAMs and promote a discussion about them – focus on systemic toxicity
- Showcase one way to integrate the presented NAMs in decision making using a real case industry application to inform a human-relevant safety decision
- To unpack our thought process whilst preparing the case study – truly end to end risk assessment, from problem formulation to safety decision

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 to consumers"

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

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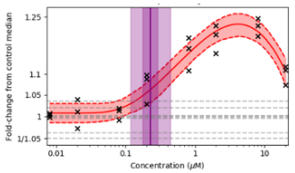
Approach to this Next Generation Risk Assessment – Protection of human health

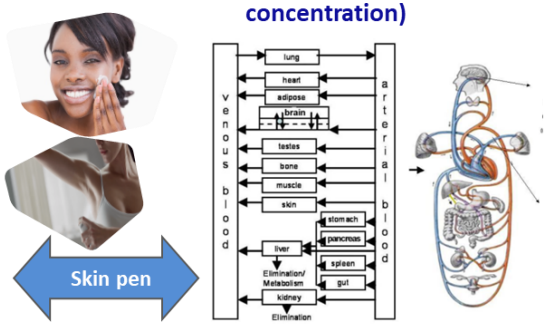
Point of departure (POD) derived from concentration-response data



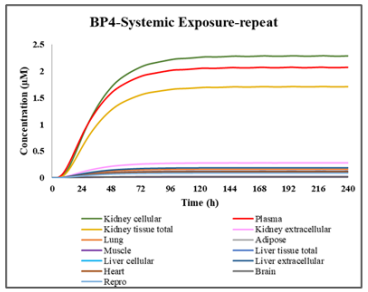
Systemic toolbox of assays (NAMs) which cover a broad biological space – **measurements of bioactivity**

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

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

Case study

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

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

Objective of the case study:

- **To assess whether a tiered NGRA approach is sufficiently protective and also useful to answer a real-life question**

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

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Benzophenone-4 (BP-4) case study: rules & assumptions

- For the purposes of this exercise, it has been assumed that **no *in vivo* animal data exist on the ingredient**
- Focus on **systemic toxicity**
- **Stand-alone illustration of how to assess systemic toxicity effects** (not including genetic toxicity) **using NAMs**

Overall approach for Benzophenone-4 (BP-4)

Gathering information

- Identified use scenario
- Identified molecular structure
- Collected existing data
- Route of exposure, habits & practises Literature, databases, In silico QSARs

Module 1 – Exposure estimation

Estimate systemic exposure concentration (SEC) (plasma C_{max})

Hypothesis Generation

Generic Core tools[1]

Module 2 – Bioactivity characterisation

- Broad suite of assays and analysis used as part of the systemic toolbox (Cell stress panel, pharmacological profiling, transcriptomics)
- Tools to address specific risk assessment questions

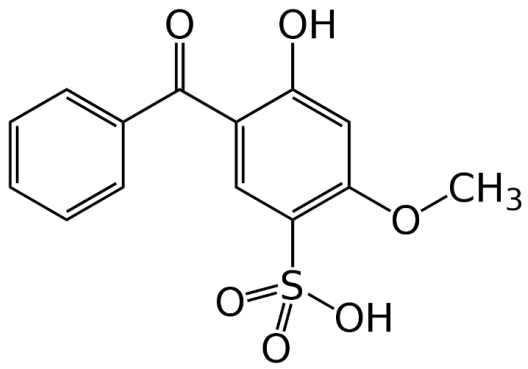
Module 3- Risk characterisation

Calculation of Bioactivity-Exposure ratio (BER). Assessment based on lowest of POD_{NAM} together with weight of evidence → Risk evaluation and risk assessment documentation

[1]Middleton et al. (2022) *Toxicol Sci* (https://doi.org/10.1093/toxsci/kfac068)

Gathering information: Use scenario and molecular structure

- Benzophenone-4 (CAS No. 4065-45-6; EC No. 223-772-2) has been used up to 5% in Europe in cosmetics for decades as an ultraviolet (UV) filter and provides protection of the skin and hair from the harmful effects of the sun.
- Benzophenone-4 is water soluble, given the presence of a sulphate group in its chemical structure and an anion at physiological pH
- It is also used as a product protectant at much lower % inclusion levels as a UV stabiliser protecting cosmetic formulations against chemical breakdown by sunlight
- The specific use scenario of this case study is for dermal application of a leave-on sunscreen body lotion product containing benzophenone-4 at 5% w/w



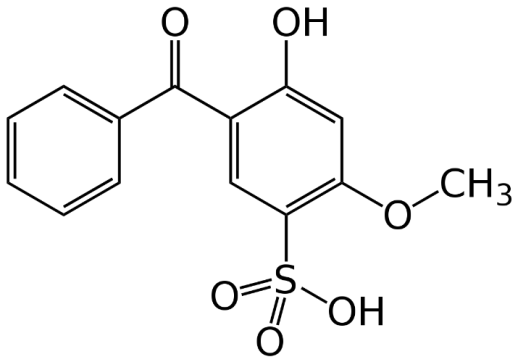
Gathering information: Alerts from *in silico* tools

- **DEREK Nexus** likely toxicity based on chemical structure
- **METEOR Nexus** possible biotransformation based on chemical structure
- **OECD QSAR Toolbox.** possible mechanisms of action
- **TIMES** likelihood of skin sensitisation of the parent and metabolites
- **OPERA** physchem, environmental fate, range of human-relevant toxicity endpoints
- **VEGA** physchem, human-relevant toxicity endpoints

AFSA training on predictive chemistry: https://youtu.be/rLWaSgGFGCI

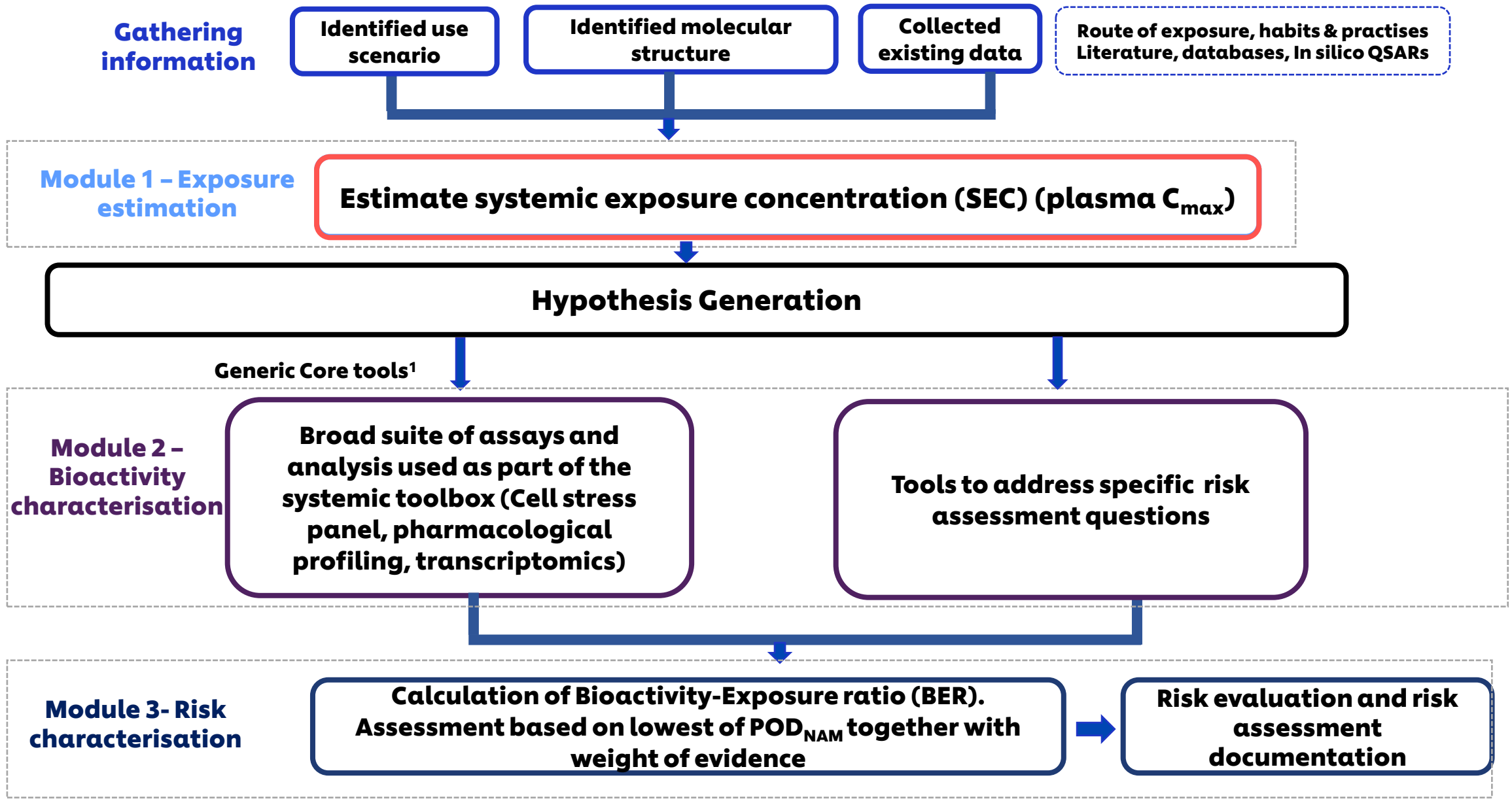
Gathering information: Alerts from *in silico* tools

- **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, non-DART toxicant, 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.



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

Overall approach for Benzophenone-4 (BP-4)



[1]Middleton et al. (2022) *Toxicol Sci* (https://doi.org/10.1093/toxsci/kfac068)

Module 1: Exposure assessment
From applied dose to internal concentrations

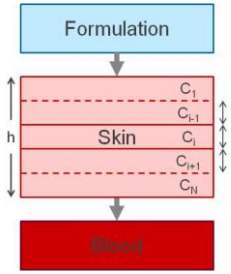
External dose

- **Route of exposure**
- **Consumer use (Habits & Practices)**
- **Applied dose (external concentration)**

ADME parameters

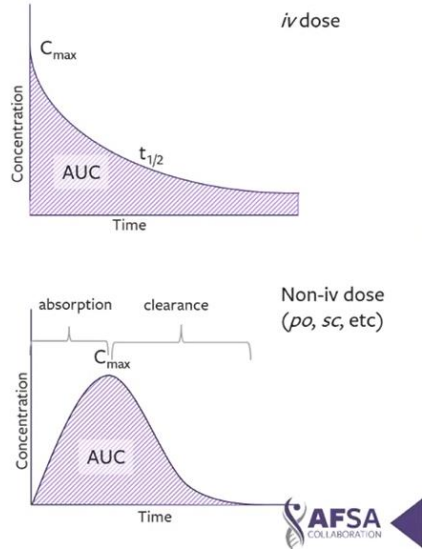
Absorption
Distribution
Metabolism
Elimination

- **Skin penetration**
- **Phys-chem properties**
- **Hepatic clearance**
- **Fraction unbound**
- **Blood:plasma ratio**



Kinetic profile of chemical

Physiologically-based kinetic (PBK) modelling – Internal concentration (plasma, urine, organ-level)

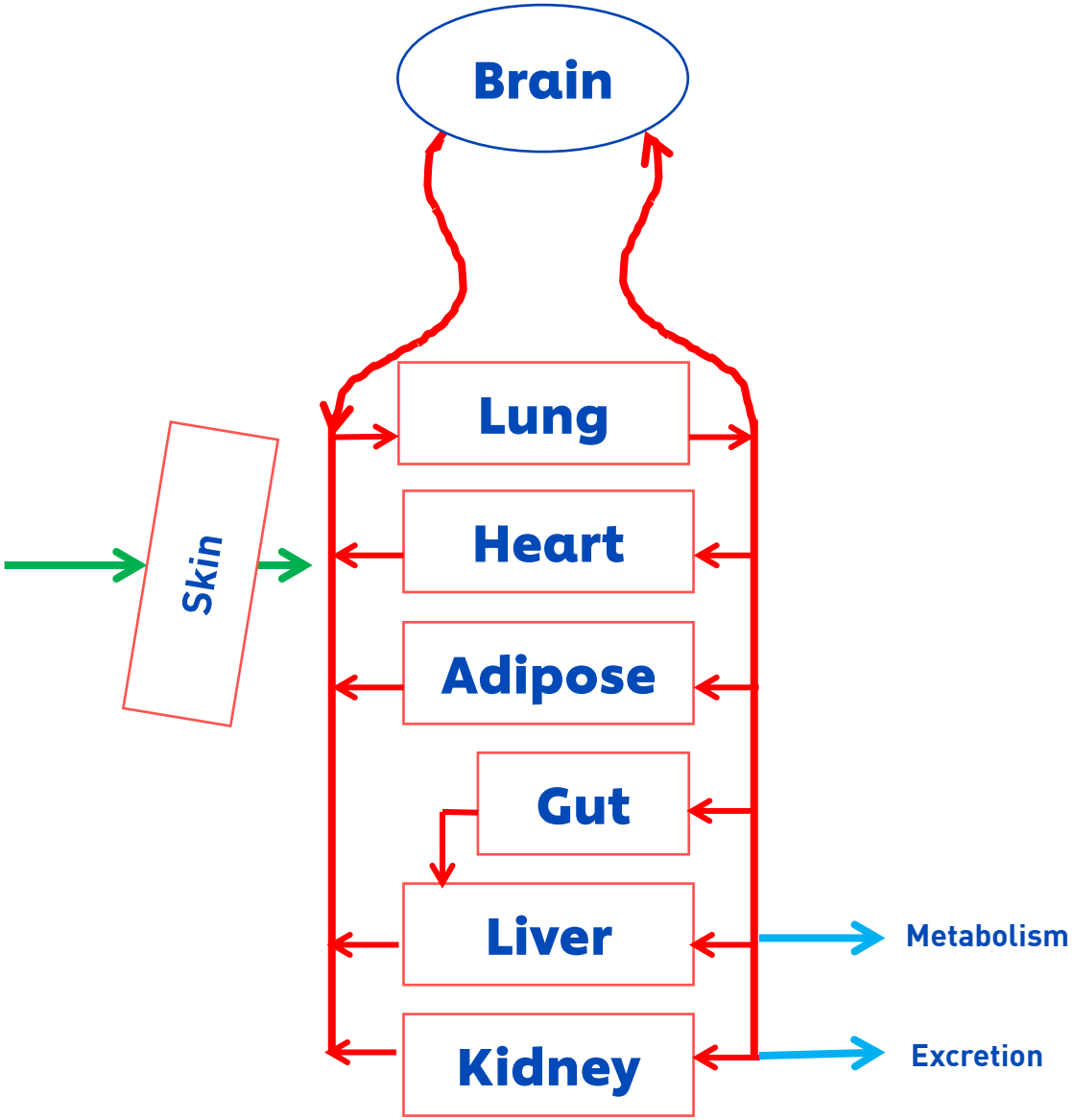


Images from: AFSA training module "Dosimetry (Internal Exposure)",2022

https://www.afsacollaboration.org/sciencex_event/dosimetry-internal-exposure-ivive/

Module 1: Exposure assessment: What is PBK modelling?

- Mathematical description of interconnected compartments representing the human body
- Describe ADME (Absorption, Distribution, Metabolism, and Excretion) properties of a chemical within the body
- Prediction of concentration in blood, plasma, and tissues over time
- Can model an individual or a population



Bayer Technology Services PK-SIM®

SIMCYP real solutions from virtual populations

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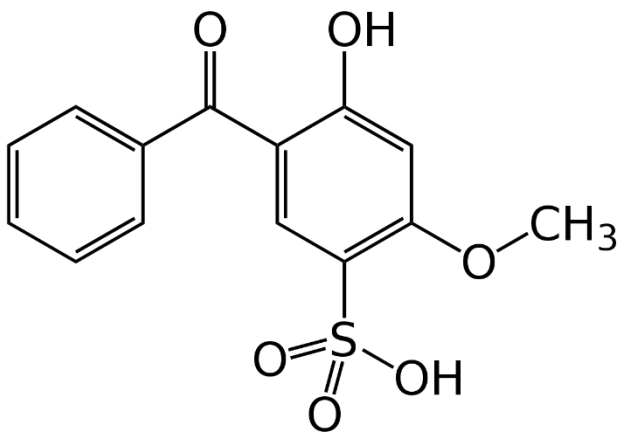
PBK modelling inputs– Exposure scenario, target individual/population, ADME parameters



Exposure scenario

- 5% in Sunscreen product,
- 18g/day, two times, 9g/application,
- On body and face 17500cm^2 (total body area)

Physiological parameters

- Adult female, 30 years old, 60 kg (SCCS NoG 12th revision)
- PEAR (Population Estimates for Age-Related -Physiology™) was used to calculate organ weights, volumes, perfusions, and tissue-plasma partition coefficients for the 30 year old, 60 kg bodyweight person.

ADME In silico & data generation in vitro

- Dermal absorption (OECD TG 428)
- Blood to plasma ratio
- Plasma protein binding
- Metabolic stability (cryopreserved primary human hepatocytes)

Moxon et al. 2020. Toxicology in Vitro, Volume 63, 104746.

PBK modelling inputs – ADME results

Main observations:

In silico

- **BP-4 was predicted to be cleared via liver metabolism (ECCS classification, Varma et al 2015)**
- **BP-4 was predicted to be substrate of several transporters by ADMET predictor**

Experimental

- **Very low skin penetration**
- **BP-4 stable in human hepatocytes. Hepatic intrinsic clearance <2.5L/h (Below LOQ)**

Conclusion: Conflicting data between in silico and experimental

Clarify hepatic clearance and understand the route of elimination

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Hepatic clearance follow up: confirming the low permeability and the lack of metabolism

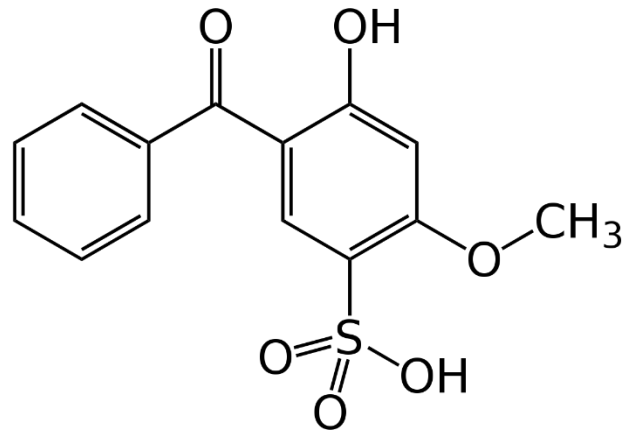
Human liver S9 incubation:

No metabolism of parent compound

- BP-4 is not a substrate of CYP enzymes
- High confidence that liver clearance is negligible (set to 0 in PBK).

Parallel artificial membrane permeability assay (PAMPA) assay:

Very low permeability



Next steps: Understanding chemical organ distribution and renal clearance

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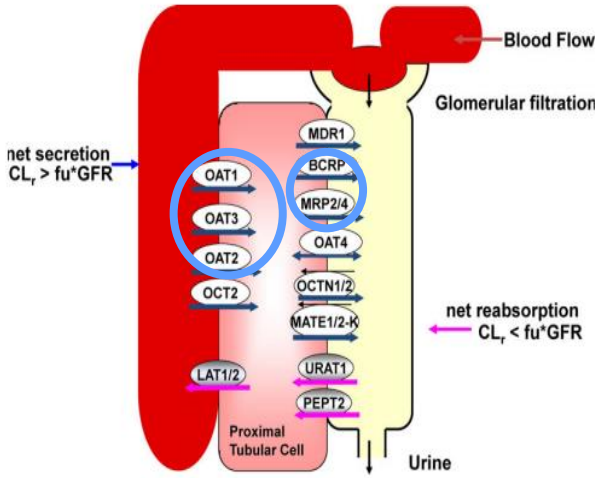
Understanding chemical organ distribution and renal clearance

1

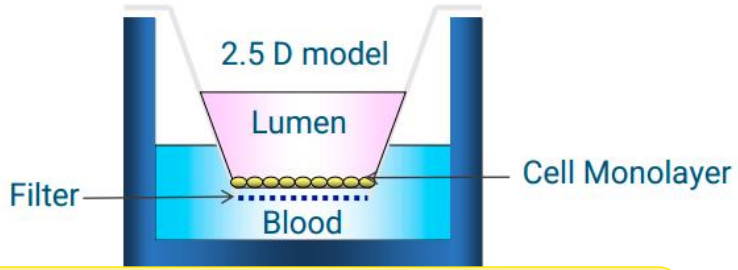
Transporter studies in transfected kidney cell



- Substrate of the influx transporters, **OAT1, OAT2, OAT3** and a substrate of the efflux transporters, **BCRP and MRP4.**
- All these transporters are expressed in the kidney, although **OAT-2, BCRP and MRP4 are expressed both in kidney and liver**

2

Transporter studies in freshly isolated kidney proximal tubule cells monolayer (aProximate™).

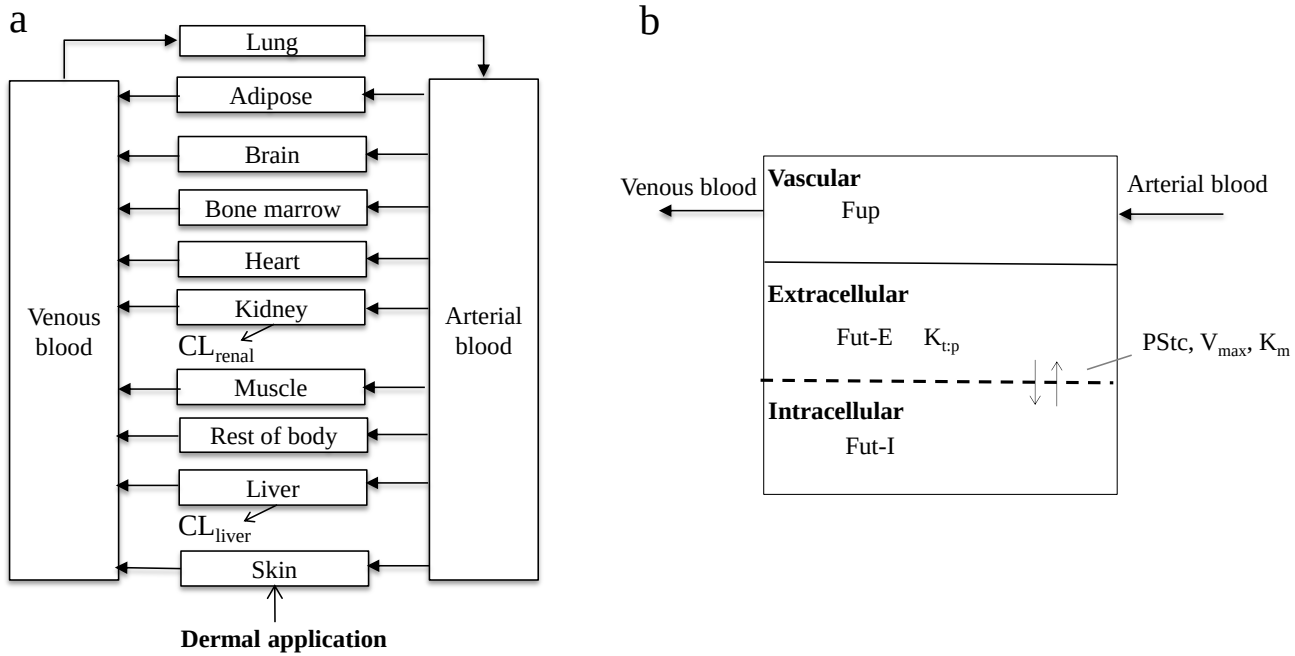


B-A →blood to urine →active secretion
A-B → urine to blood →reabsorption

- Transport in the proximal tubule cells is **equally efficient in both direction**s leading to no net movement

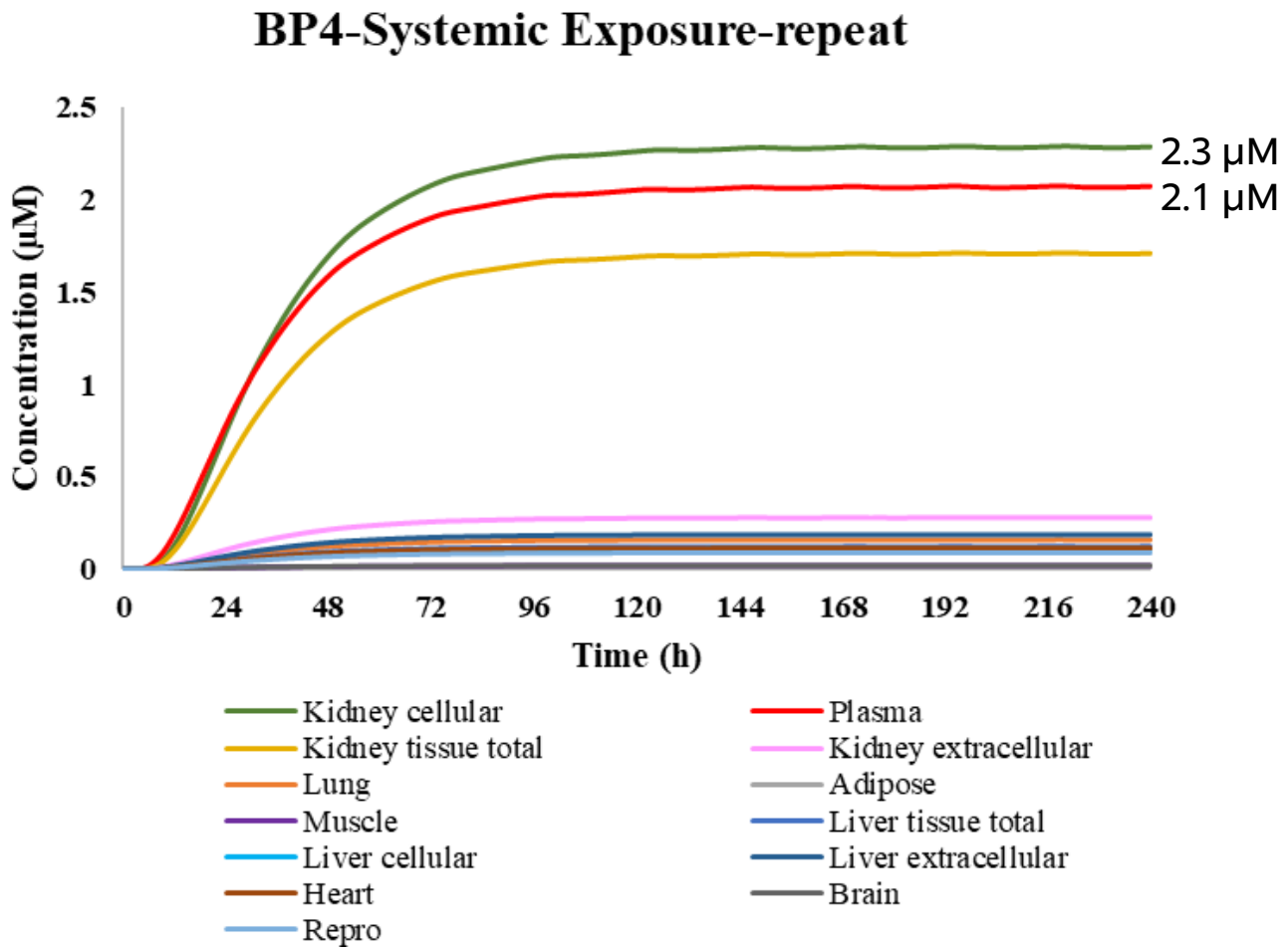
Update PBK model – choose the most conservative assumptions

- Set BP-4's distribution to each compartment to be modelled as permeability-limited
- Liver clearance set to 0
- Active transport in the liver was modelled by incorporating kinetic parameters for the transporters (OAT-2, BCRP and MRP4).
- GFR*Fup was used to calculate renal excretion of benzophenone-4, accounting for filtration only to be conservative



Human PBK model structure for BP4

Internal concentration: Deterministic PBK model simulation of C_{max} for an adult female (30 years old, 60 kg)



Benzophenone-4 concentrations in plasma and different tissues after repeated exposure of body lotion 18g/day, i.e., 9g twice per day for a period of 10 days, with 5% benzophenone-4, on the whole body.

To summarize BP-4's kinetic behavior in the human body:

- Overall, upon dermal absorption only a small amount of BP-4 enters systemic circulation, after which BP-4 remains unchanged due to negligible liver clearance.
- It has low tissue distribution due to low partitioning and limited passive diffusion of cell membranes (negatively charged at physiological pH).
- It can be taken up into the kidney and then excreted to urine via active transport and can be reabsorbed back to into the bloodstream, however, due to no preferred direction of movement, glomerular filtration determines the overall renal excretion rate.
- BP-4 can also move into and then out of the liver cells via active transport (OAT2).

Breakout discussion I

In your groups discuss the following:

1. How would these in silico predictions inform the next steps in the risk assessments? (i.e. follow up in vitro testing)
2. How confident are you in the predicted values of plasma Cmax?
3. How would you increase the confidence in the exposure prediction? (i.e. What other information would you like to have?)
4. How would these exposure results inform your next steps in the risk assessment?

slido

https://app.sli.do/event/5BhpcwjEHnYGKMdJ3NfMae

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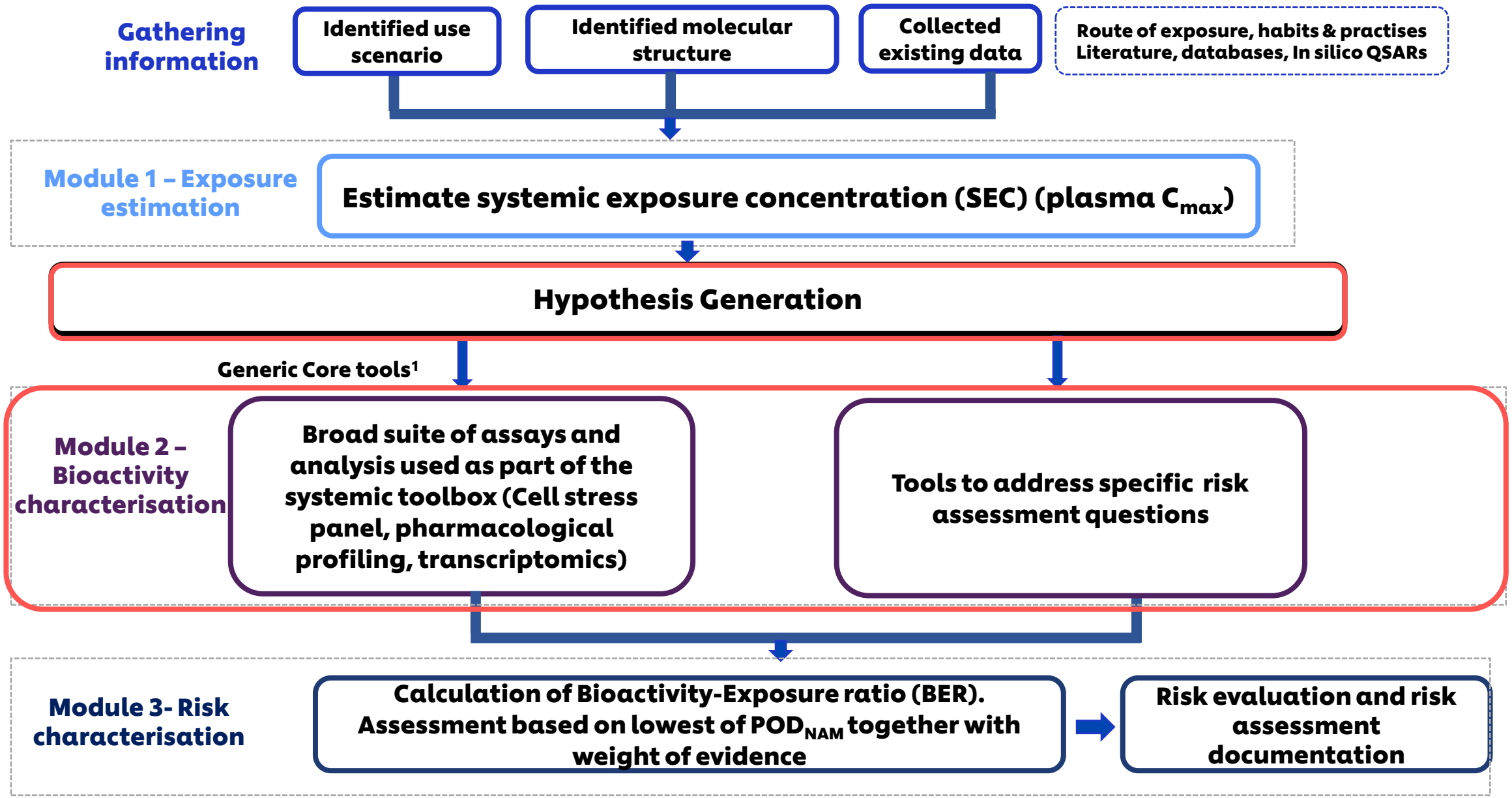
Overall approach for Benzophenone-4 (BP-4)



[1]Middleton et al. (2022) *Toxicol Sci* (https://doi.org/10.1093/toxsci/kfac068)

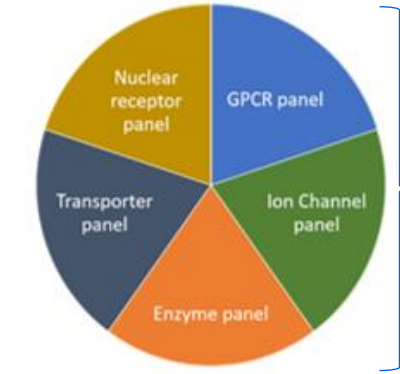
Hypothesis Generation

1) **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.**
2) In silico tools predicted binding to estrogen receptor.
3) PBK model indicated that the concentration of BP-4 is higher in the kidney than in any other organ, therefore a relevant kidney cell model was included in the testing strategy.

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

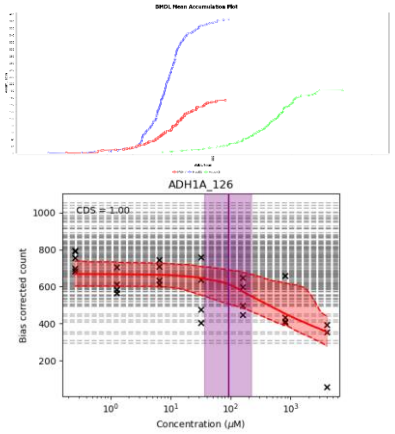
Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

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
- 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

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

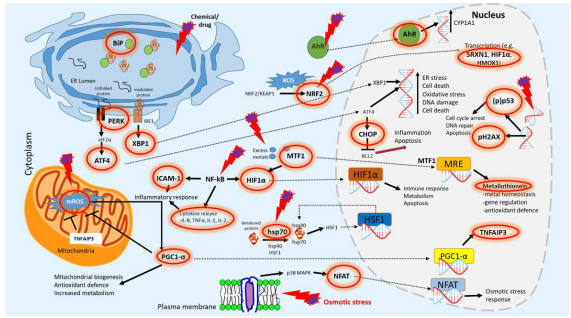


Image kindly provided by Paul Walker (Cyprotex)

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

High Throughput Transcriptomics (HTTr) applied as a broad nontargeted biological screen

- HTTr provides information **genome-wide biological perturbations**
- **Concentration-response** HTTr experiments can provide **potency estimates** for the concentrations of chemicals that produce perturbations in cellular response pathways
- **TempO-Seq technology** is the method adopted by the US EPA, Health Canada and in the APCRA case studies.

Experimental design for case study:

- Use of full human gene panel ~ 21k
- 24 hrs exposure, 7 concentrations
- 4 cell lines: HepG2 (OAT2), HepaRG (OAT2) and MCF7 (OAT1) and primary proximal tubule cells (PTCs; (aProximate™))

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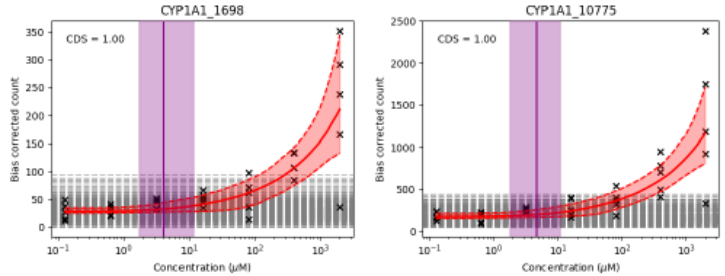
Harrill et al. Toxicol Sci (2021) 181(1):68-89

BioClavis®

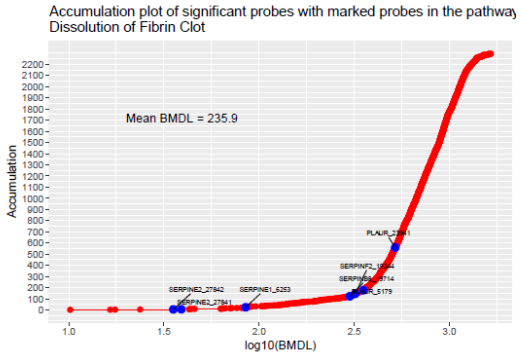
High Throughput Transcriptomics (HTTr) applied as a broad nontargeted biological screen

Data Analysis

- For the purposes of this case study, dose-response analysis and point of departure (POD) determination was performed using 2 different methods:
  - **Global POD (BIFROST method):** Estimate of the highest nominal concentration of test substance at which there is no bioactivity.



  - **Pathway average Bench Mark Dose Lowest (BMDL):** Average of all gene level BMDLs for genes within a pre-defined pathway using BMDExpress2. POD defined here is the lowest observed concentration that shows significant pathway perturbation.



Cell stress panel- 10 stress pathways responsible for cell homeostasis

cyprotex AN EVOTEC COMPANY

- **~<u>10 Stress Pathways</u>: mitochondrial toxicity, Oxidative Damage, DNA damage, Inflammation, ER stress, Metal stress, Heat Shock, Hypoxia, Cell Health**

- HepG2 cells
- 36 Biomarkers;
- 24h exposure duration
- 8 Concentrations
- Dose response analysis and derivation of **Global POD by the BIFROST method**[1]

OXFORD SOT Society of Toxicology
academic.oup.com/toxsci

TOXICOLOGICAL SCIENCES, 2020, 1–23
doi: 10.1093/toxsci/kfaa054
Advance Access Publication Date: May 6, 2020
Research article

FEATURED

Identifying and Characterizing Stress Pathways of Concern for Consumer Safety in Next-Generation Risk Assessment

Sarah Hatherell,* Maria T. Baltazar,* Joe Reynolds,* Paul L. Carmichael,* Matthew Dent,* Hequn Li,* Stephanie Ryder,† Andrew White,* Paul Walker ,† and Alistair M. Middleton*,1

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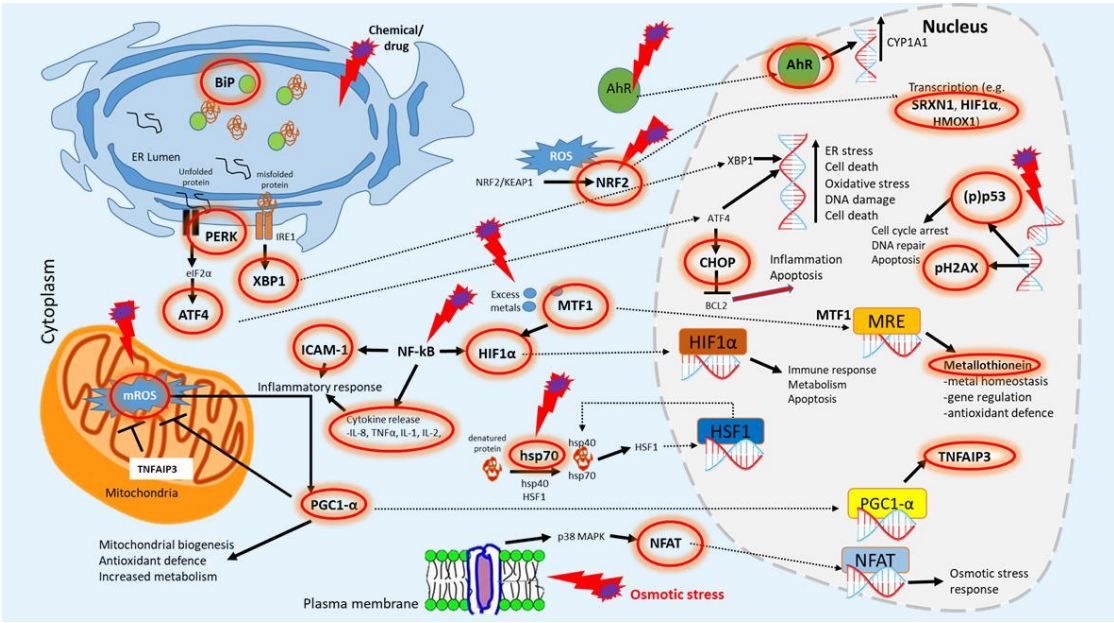

[1]Middleton et al. (2022) *Toxicol Sci* (https://doi.org/10.1093/toxsci/kfac068)

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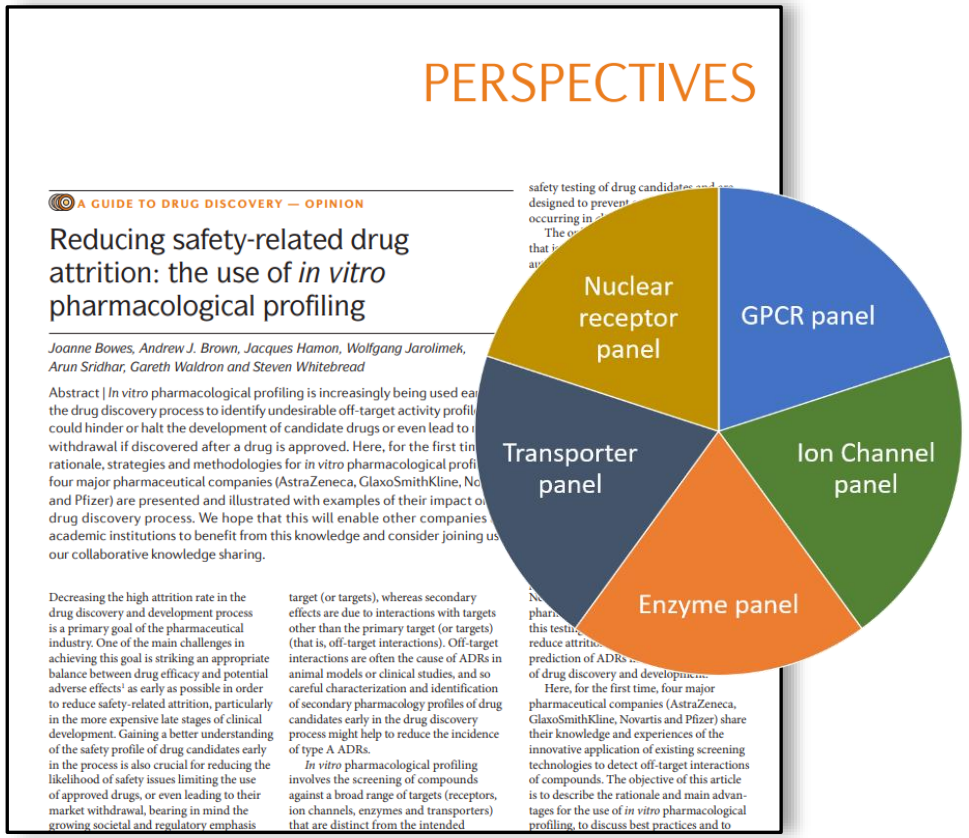
In vitro pharmacological profiling- currently 79 targets



PERSPECTIVES

A GUIDE TO DRUG DISCOVERY — OPINION

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

Abstract | *In vitro* pharmacological profiling is increasingly being used ea... the drug discovery process to identify undesirable off-target activity profil... could hinder or halt the development of candidate drugs or even lead to ... withdrawal if discovered after a drug is approved. Here, for the first tim... rationale, strategies and methodologies for *in vitro* pharmacological profi... four major pharmaceutical companies (AstraZeneca, GlaxoSmithKline, No... and Pfizer) are presented and illustrated with examples of their impact o... drug discovery process. We hope that this will enable other companies ... academic institutions to benefit from this knowledge and consider joining us ... our collaborative knowledge sharing.

Decreasing the high attrition rate in the drug discovery and development process is a primary goal of the pharmaceutical industry. One of the main challenges in achieving this goal is striking an appropriate balance between drug efficacy and potential adverse effects[1] as early as possible in order to reduce safety-related attrition, particularly in the more expensive late stages of clinical development. Gaining a better understanding of the safety profile of drug candidates early in the process is also crucial for reducing the likelihood of safety issues limiting the use of approved drugs, or even leading to their market withdrawal, bearing in mind the growing societal and regulatory emphasis

target (or targets), whereas secondary effects are due to interactions with targets other than the primary target (or targets) (that is, off-target interactions). Off-target interactions are often the cause of ADRs in animal models or clinical studies, and so careful characterization and identification of secondary pharmacology profiles of drug candidates early in the drug discovery process might help to reduce the incidence of type A ADRs.

In vitro pharmacological profiling involves the screening of compounds against a broad range of targets (receptors, ion channels, enzymes and transporters) that are distinct from the intended

safety testing of drug candidates ... designed to prevent ... occurring in ... The ... that i... ar...

... this testi... reduce attrit... prediction of ADRs ... of drug discovery and develop...

Here, for the first time, four major pharmaceutical companies (AstraZeneca, GlaxoSmithKline, Novartis and Pfizer) share their knowledge and experiences of the innovative application of existing screening technologies to detect off-target interactions of compounds. The objective of this article is to describe the rationale and main advantages for the use of *in vitro* pharmacological profiling, to discuss best practices and to

- Panel developed by the pharmaceutical industry and used during early drug discovery to predict, assess and minimise/avoid risk of potential off-target adverse drug reactions.
- Initial panel of 44 targets identified to be related to adverse health outcomes[1]
- Cosmetics Europe/LRSS working group added 29 additional targets selected via literature review of 78 targets found in at least two separate sources (secondary pharmacology reviews, legacy data from companies) [2,3,4]

eurofins | DISCOVERY

1. Bowes J et al 2012. Nat Rev Drug Discov;11(12):909-22.
2. Lynch JJ et al., 2017 Pharmacol Toxicol Methods;87:108-126.
3. Smit IA et al., 2021 Chem Res Toxicol;34(2):365-384.
4. Letswaart R et al., 2020 EBioMedicine;57:102837

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Hypothesis Generation

1) 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.

2) **In silico tools predicted binding to estrogen receptor.**

3) **PBK model indicated that the concentration of BP-4 is higher in the kidney than in any other organ, therefore a relevant kidney cell model was included in the testing strategy.**

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Module 2: Tools to address specific risk assessment questions

2. In silico prediction for estrogen binding

EATS activity: estrogenic, androgenic, thyroidogenic and steroidogenesis

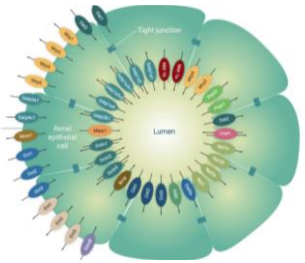
- CALUX bioassays to measure transcriptional activation and binding assays: TTR-TRβ- and hTPO
- U2-OS incorporating the firefly luciferase reporter gene coupled to Responsive Elements (REs)
- 12 concentrations. Calculation of AC50, LOEC and NOEC

3. Benzophenone-4 concentration was predicted to be higher in the kidney than any other organ

4. Cell models in the toolbox have limited expression of the relevant transporters

Renal Toxicity

Renal biomarkers (3 donors, duplicate per donor), 8 concentrations, 24h and 72h timepoints:



- 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

Newcells aProximate™ platform

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

Results from the key NAMs- 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 PTC cells - BIFROST (gene level PTC, 320 µM) and BMDL (pathway level PTC, N/A)

Cell Stress Panel

- Global POD_{NAM} = 140 µM

In vitro Pharmacological profiling

- Tested up to 10 µM
- ~79 targets compiled by Cosmetics Europe Safety pharmacology WG
- **No hits**

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 (Cisplatin and Omeprazole gave expected dose-response at 72-h)

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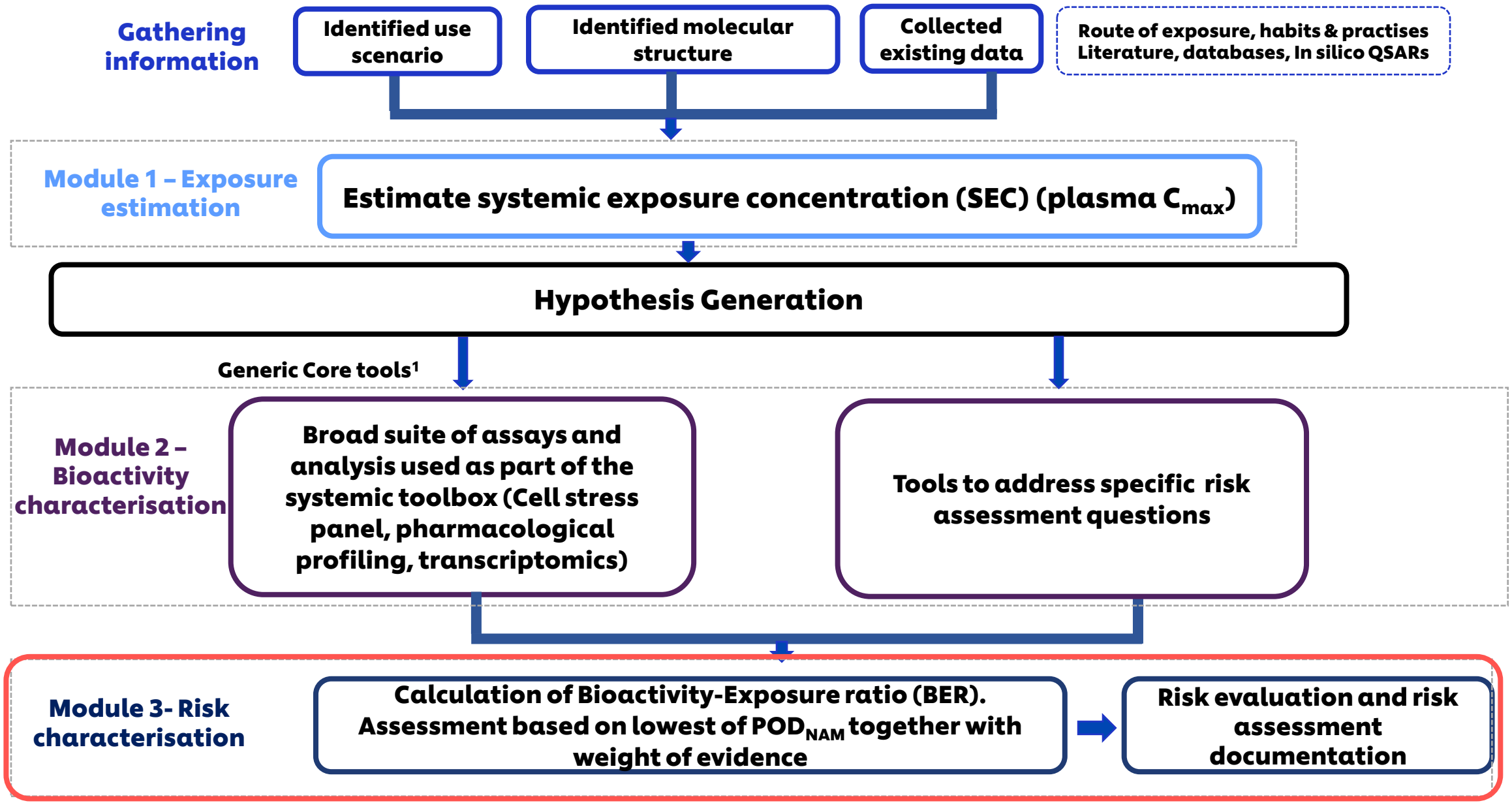
COSMETICS EUROPE LRSS

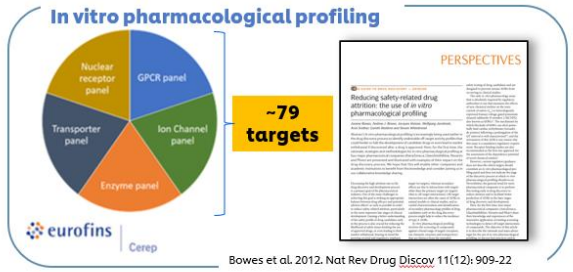
Overall approach for Benzophenone-4 (BP-4)



[1]Middleton et al. (2022) *Toxicol Sci* (https://doi.org/10.1093/toxsci/kfac068)

Risk Assessment Outcome

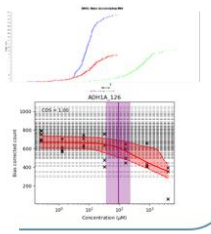
BIOACTIVITY



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

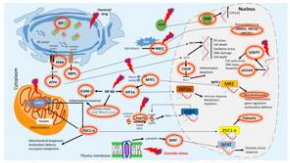
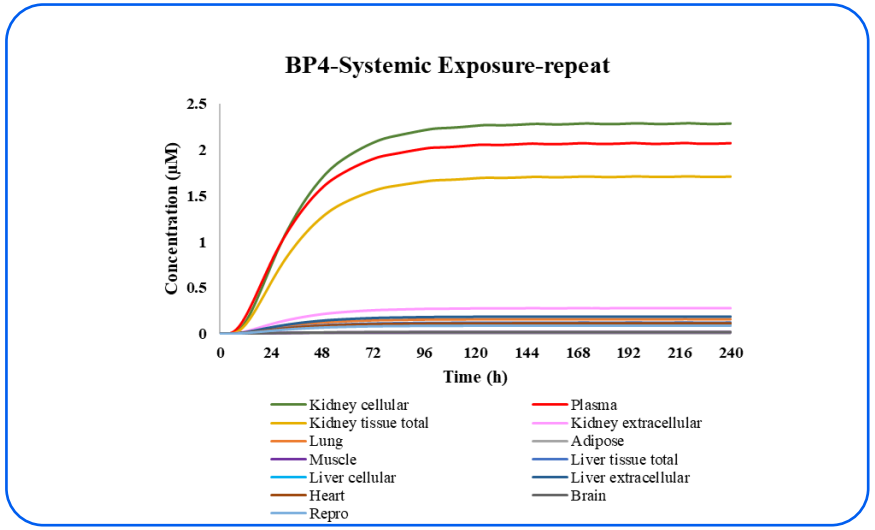


Image kindly provided by Paul Walker (Cyprotex)

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

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

Bioactivity: exposure ratio calculation: BER ranging from 2-300

Broad suit of assays

NAM	Cell type	POD_{NAM} Type	POD_{NAM} Value (µM)	BER (using C_{max} of 2.1 µM)
Cell stress panel	HepG2	Gene-based PoD	140	67
HTTr	HepG2	Gene-based PoD	4.2	2
HTTr	HepaRG	Gene-based PoD	52	25
HTTr	MCF7	Gene-based PoD	5.5	2.6
HTTr	HepaRG	Lowest pathway BMDL	530	252
HTTr	HepG2	Lowest pathway BMDL	240	114
HTTr	MCF7	Lowest pathway BMDL	330	157

Specific assays

NAM	Cell type	POD_{NAM} Type	POD_{NAM} Value (µM)	BER (using C_{max} of 2.1 µM)
Calux (hTPO-inhibition)	-	LOEC	300	143
Calux (T4 binding to TTR)	-	LOEC	630	300
Renal biomarkers (24 hr exposure)	PTC	PoD	>1000	NA
Renal biomarkers (72 hr exposure)	PTC	PoD	>1000	NA
HTTr (renal cells) (24 hr exposure)	PTC	Gene-based PoD	320	152
HTTr (renal cells) (72 hr exposure)	PTC	Gene-based PoD	320	152

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:

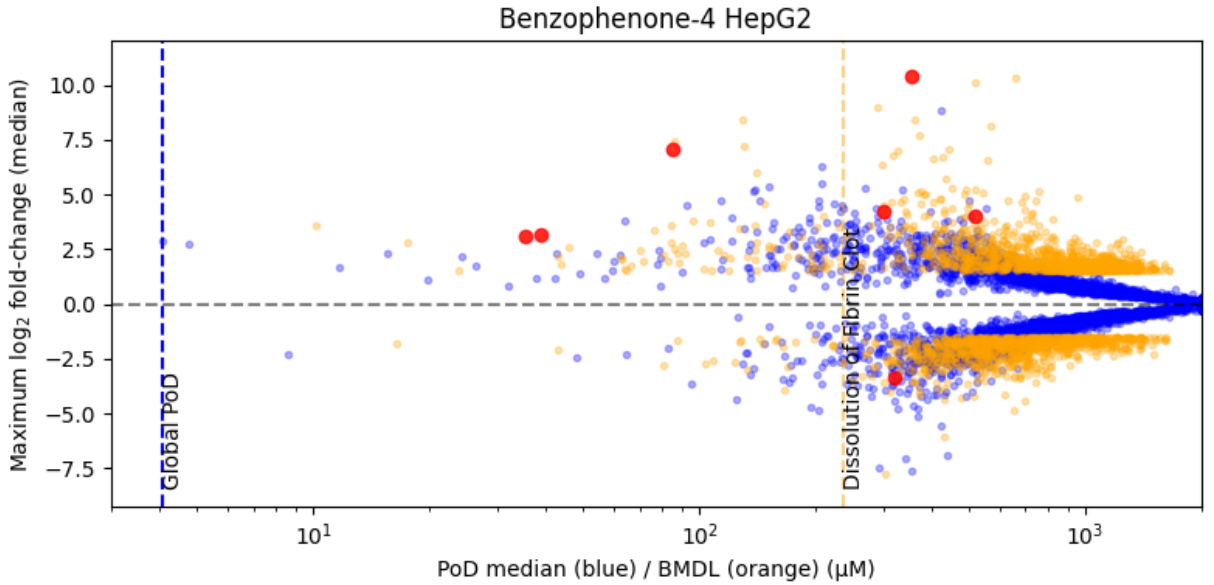
a) The in vitro measures of bioactivity provide appropriate biological coverage

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; deterministic BER of 2)
  - *Single gene change of CYP 1A1*
  - *Lowest BMDL in the same cell line is 240* µM (deterministic BER of 114)
  - This provides some assurance that the gene changes seen at 4.1 µM may be of limited toxicological significance.
- The BER calculated from the deterministic Cmax and cell stress panel global POD (the next lowest POD) was 67.



Safety assessment discussion

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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Breakout discussion 2

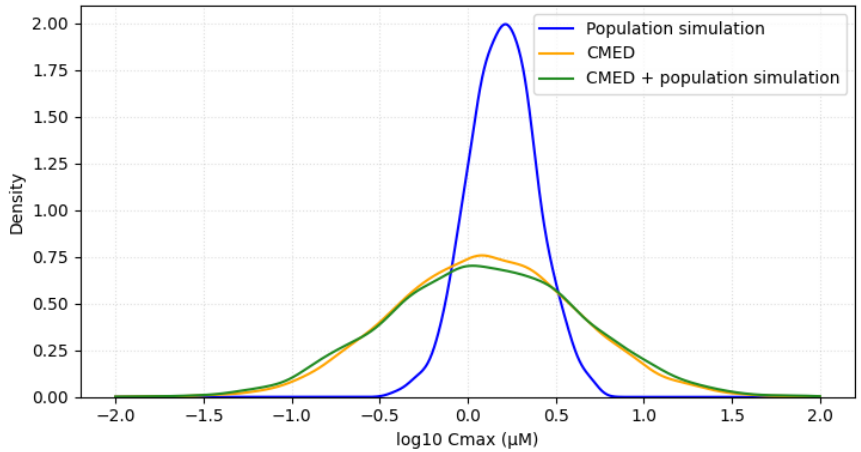
https://app.sli.do/event/5BhpcwjEHnYGKMdJ3NfMae

1. How confident are you about the use/interpretation of the bioactivity data?
2. How confident are you about making a risk assessment decision?
3. What are the main remaining uncertainties in the risk assessment?
4. What other data types/information would increase your confidence?

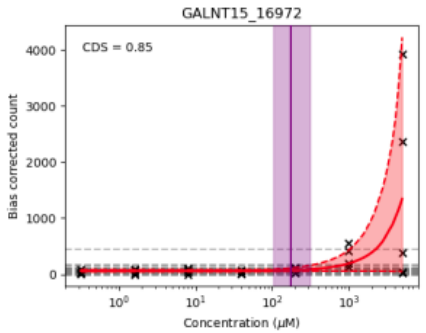
Addressing uncertainties in the safety assessment

Quantitative assessment-Probabilistic approaches for exposure & PoD determination

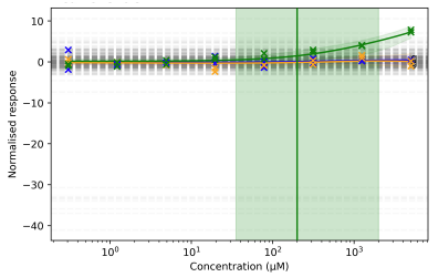
Population variability and uncertainty in model parameters



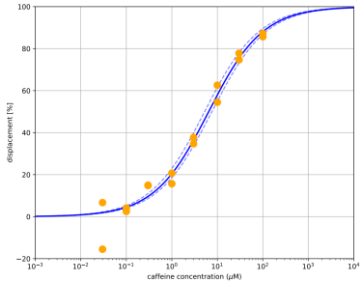
Gene expression



Cell stress panel



IPP target response



Qualitative assessment

Area	Level of certainty (rationale)	Is value likely to be an over- or under-estimate (rationale)	Impact on risk assessment decision

Areas

- Consumer exposure (applied dose)
- Identification of metabolites
- Consumer exposure (Internal dose)
- Range of biomarkers assessed
- Use of short-term tests *in vitro* to inform about risks of long-term human exposure
- Point of departure selection

Similar approach to OECD (2021): IATA for Phenoxyethanol

Area	Level of certainty (rationale)	Is value likely to be an over- or under-estimate (rationale)	Impact on risk assessment decision
Range of biomarkers assessed	**Moderate** (There is increasing evidence that POD_{NAM} obtained from the core NAMs, IPP, CSP and HTTr are protective for a range of chemicals (Middleton *et al.*, 2022) and previous case studies (Baltazar *et al.*, 2020, OECD phenoxyethanol). The hypothesis and exposure driven approach led to the inclusion of additional NAMs to investigate potential endocrine activity and kidney toxicity)	**Given the low activity of benzophenone-4 across all available assays together with its kinetic profile (low passive permeability and low organ distribution) it is considered unlikely a specific MoA exists that would affect the safety assessment**	There are remaining uncertainties regarding the protectiveness of the tools utilised for a broader range of chemistries. **Confidence could be increased by assessing how protective the range of biomarkers are for many more compounds** and whether different biomarkers are needed to ensure the *in vitro* PoD is protective compared with the *in vivo* PoD

Conclusions & reflections

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

Lowest BER= 2

BER range= 2-300

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 general 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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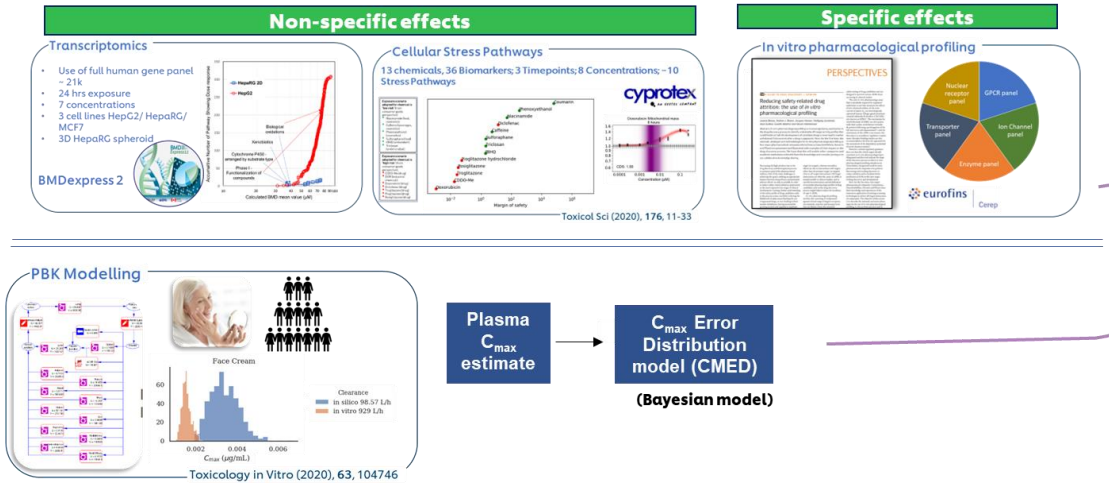
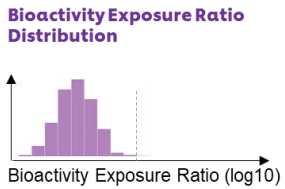
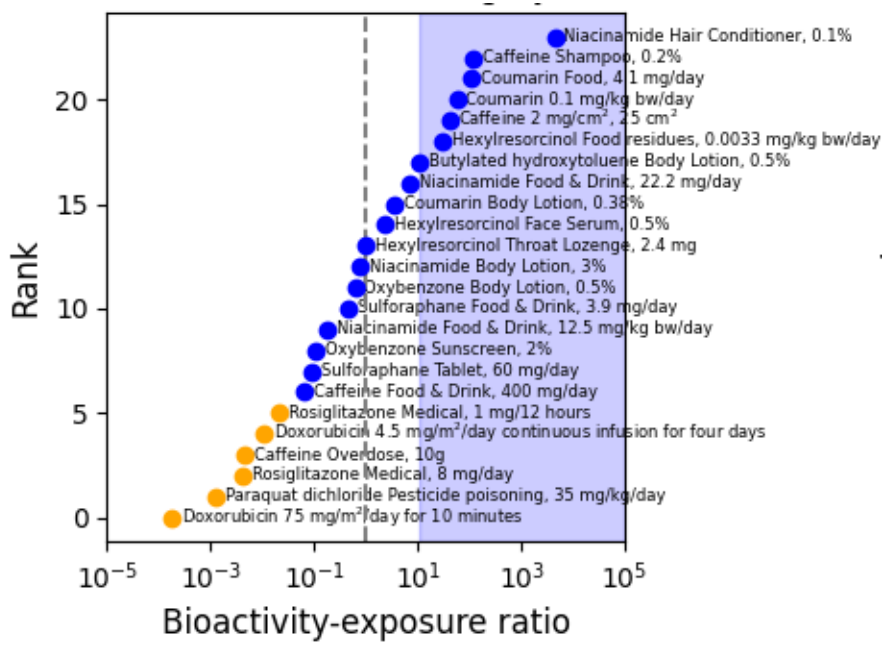
Ongoing work to build confidence in the core toolbox for Tier 1

Point of Departure determination





First pilot 10 chemicals, 24 exposure scenarios

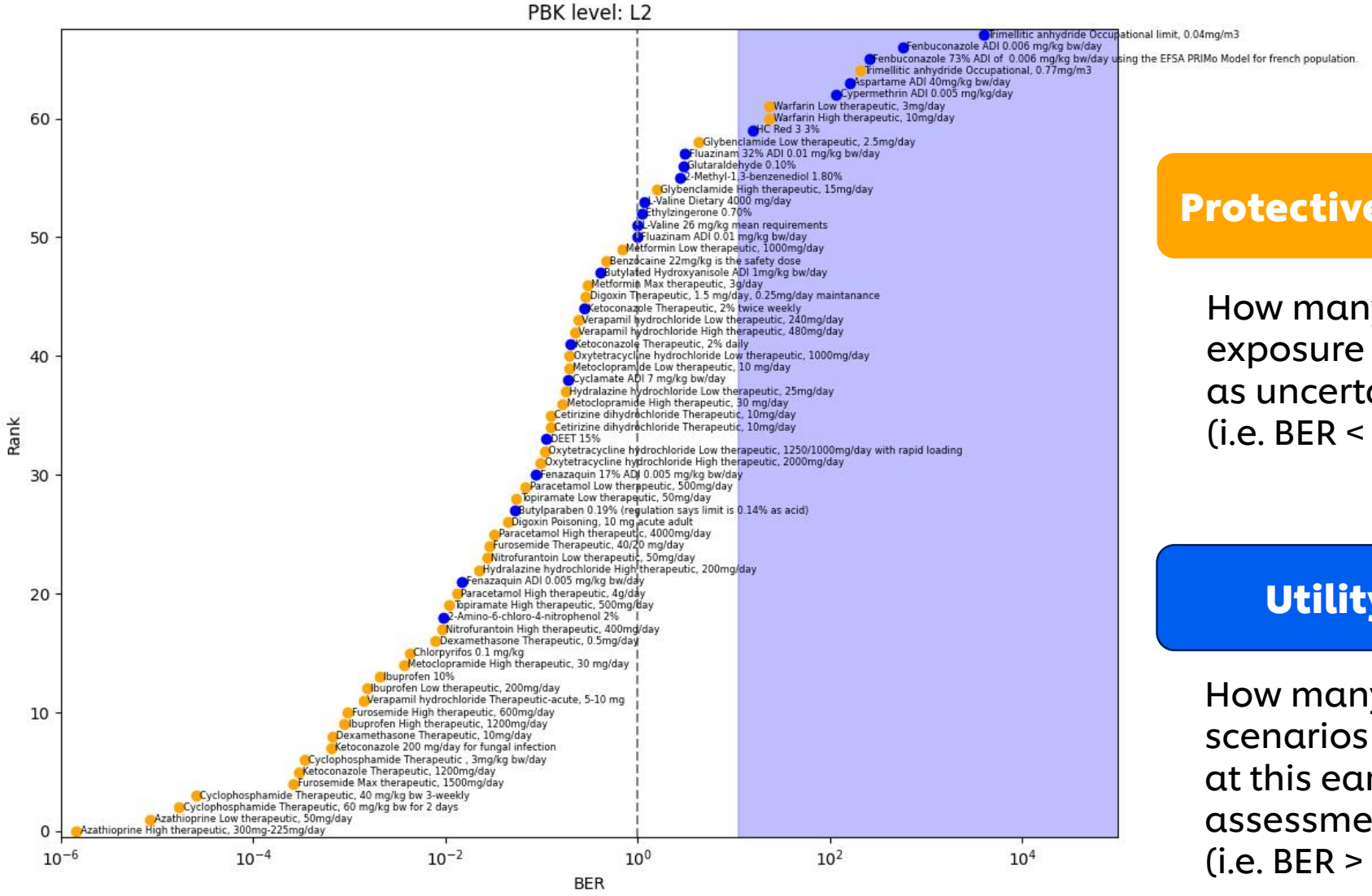


‘Low’ risk for consumers from systemic perspective

‘High’ risk for consumers from systemic perspective

Middleton et al. (2022) *Toxicol Sci* (https://doi.org/10.1093/toxsci/kfac068)

Results for a set of 38 test chemicals and 70 exposure scenarios (manuscript in preparation, Cable et al)



Protectiveness **93% (43 out of 46)**

How many of the high risk exposure scenarios are identified as uncertain/high risk
(i.e. BER < threshold)

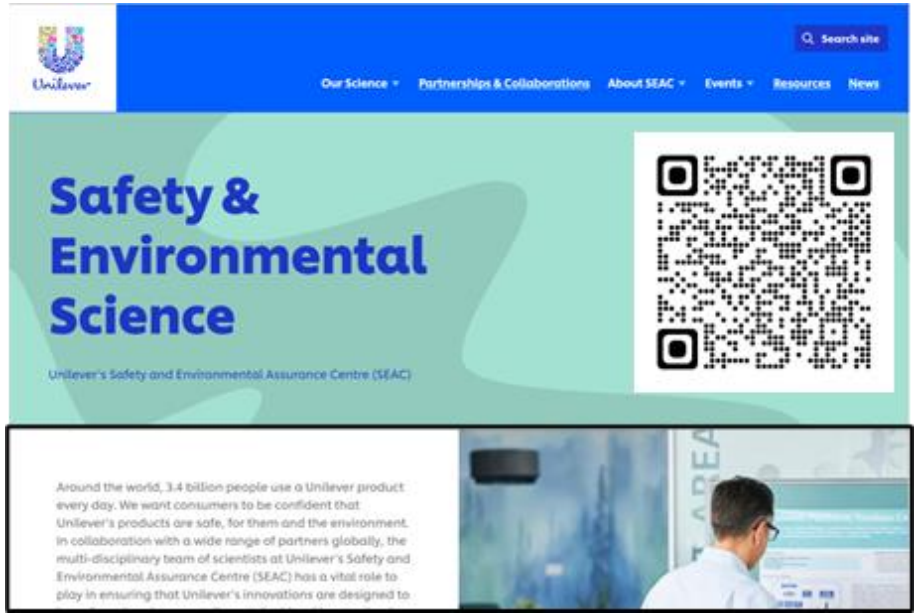
Utility **27% (6 out of 22)**

How many of the low risk scenarios are identified as low risk at this early tier stage in a risk assessment framework
(i.e. BER > threshold)

Acknowledgments

Matt Dent
Hequn Li
Sophie Cable
Nicky Hewitt
Beate Nicol
Ans Punt
Joe Reynolds
Sophie Malcomber
Sharon Scott
Jade Houghton
Predrag Kukic
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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