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

24/10/2023

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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

Principles of 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***

Dent et al 2018. Computational Toxicology Volume 7, August 2018, Pages 20-26

ICCR
9 principles of NGRA

Main overriding principles:
The overall goal is a human safety risk assessment
The assessment is exposure led
The assessment is hypothesis driven
The assessment is designed to prevent harm

Principles describe how a NGRA should be conducted:
Following an appropriate appraisal of existing information
Using a tiered and iterative approach
Using robust and relevant methods and strategies

Principles for documenting NGRA:
Sources of uncertainty should be characterized and documented
The logic of the approach should be transparently and documented

ICCR International Cooperation on Cosmetics Regulation

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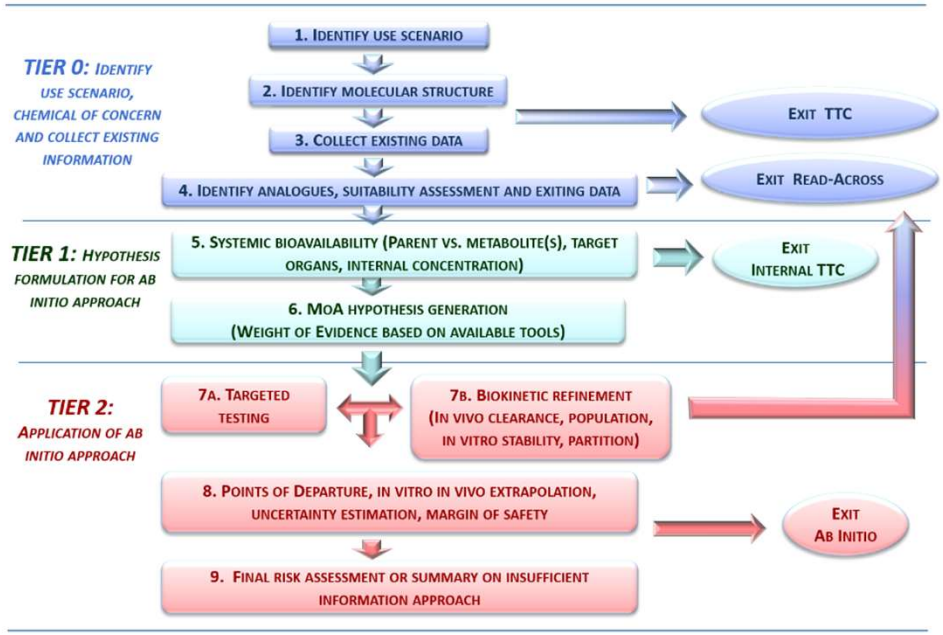
Next Generation Risk Assessment: From Principles to Application



SEURAT-1

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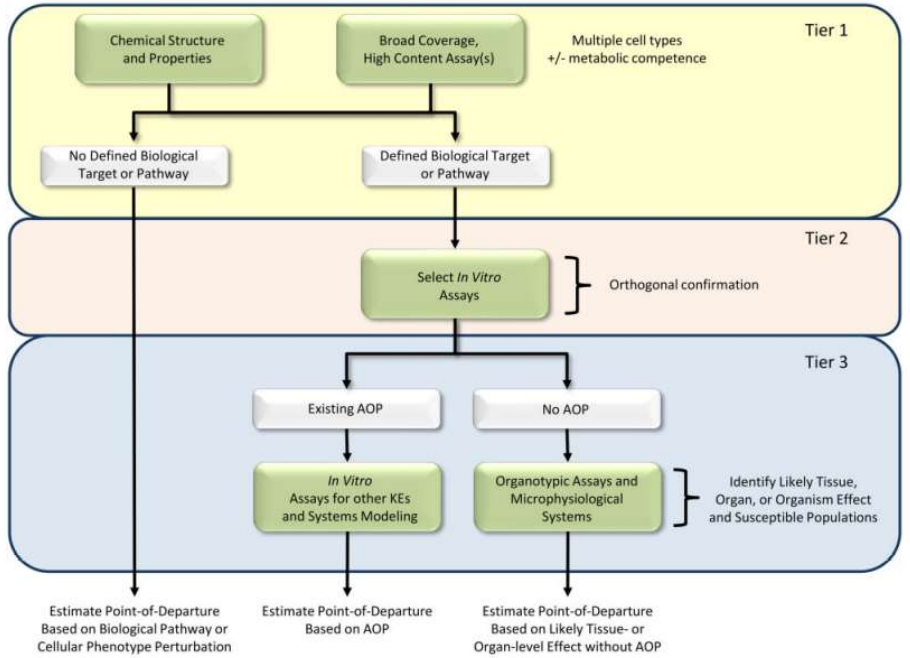


Figure 2. Tiered testing framework for hazard characterization. Tier 1 uses both chemical structure and broad coverage, high content assays across multiple cell types for comprehensively evaluating the potential effects of chemicals and grouping them based on similarity in potential hazards. For chemicals from Tier 1 without a defined biological target / pathway, a quantitative point-of-departure for hazard is estimated based on the absence of biological pathway or cellular phenotype perturbation. Chemicals from Tier 1 with a predicted biological target or pathway are evaluated Tier 2 using targeted follow-up assays. In Tier 3, the likely tissue, organ, or organism-level effects are considered based on either existing adverse outcome pathways (AOP) or more complex culture systems. Quantitative points-of-departure for hazard are estimated based on the AOP or responses in the complex culture system.

OXFORD SOT Society of Toxicology
www.toxsci.oxfordjournals.org

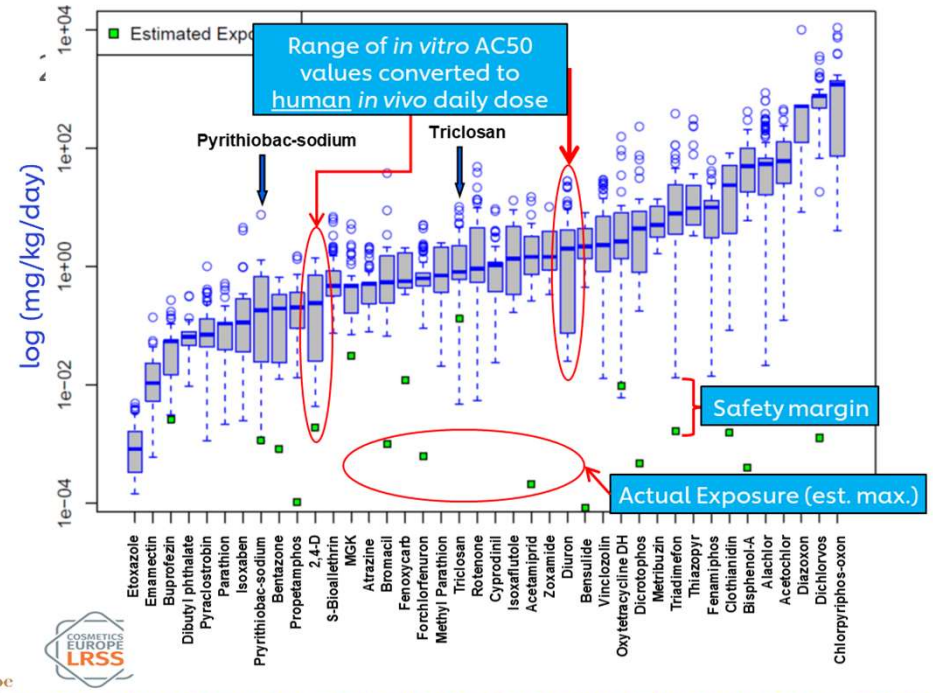
TOXICOLOGICAL SCIENCES, 169(2), 2019, 317–332

FORUM

The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency

Protection not prediction concept – This NGRA strategy

Distributions of Oral Equivalent Values and Predicted Chronic Exposures



Graph from Rusty Thomas EPA, with thanks. Rotroff *et al* (2010) Toxicological Sciences , **117**, 348-358

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

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

At no point does NGRA attempt to predict the results of high dose toxicology studies in animals.

BP-4 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:

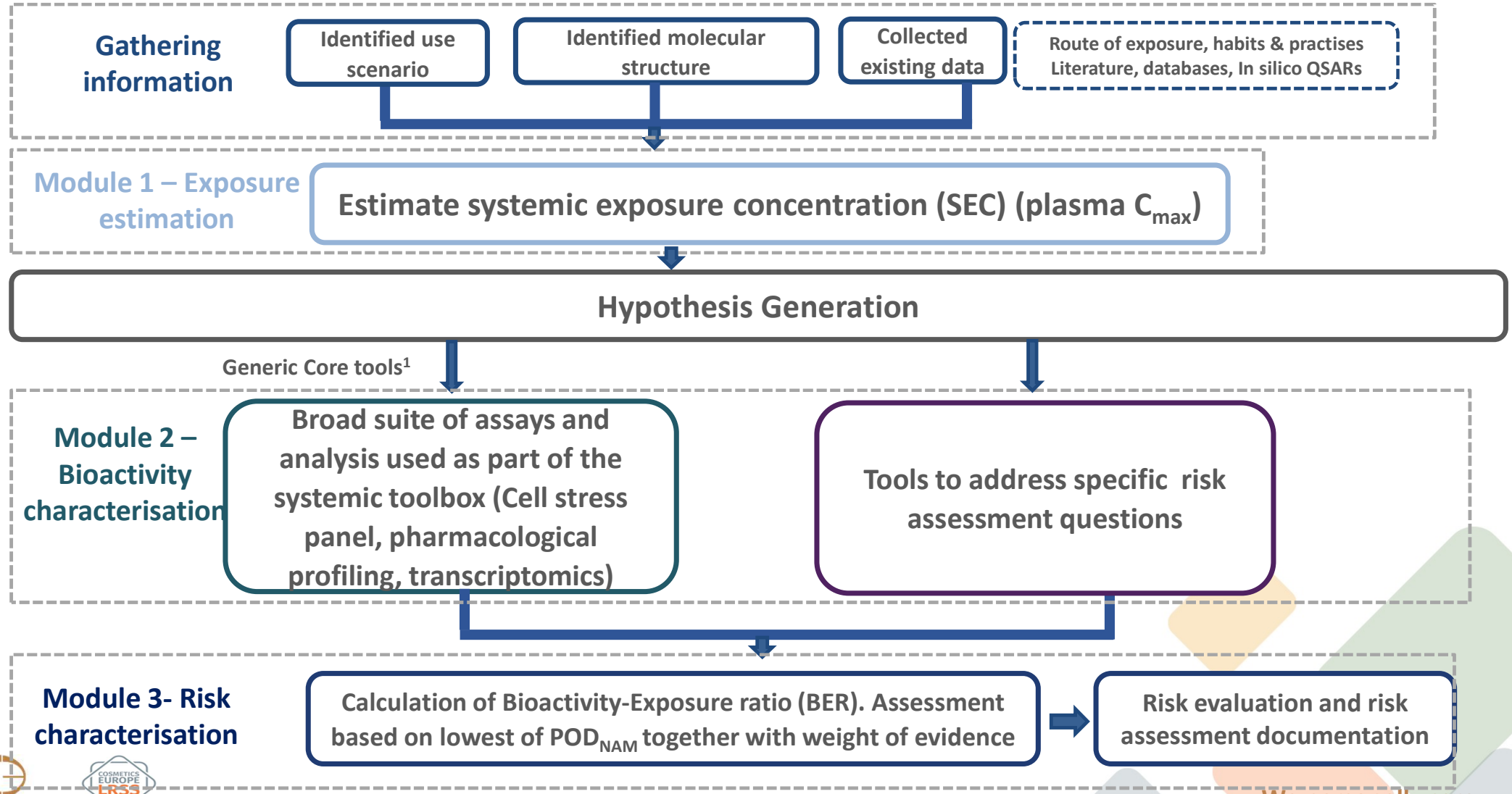
- **To assess whether a tiered NGRA approach is sufficiently protective and also useful to answer a real-life question**

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

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

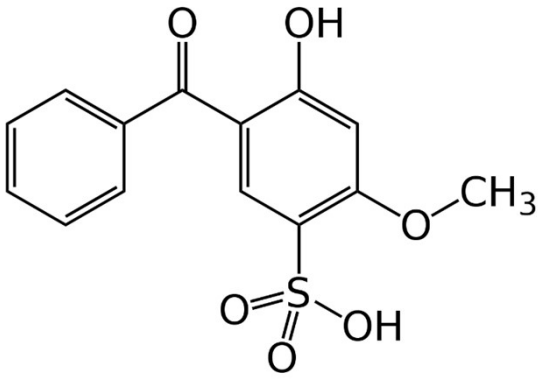
- For the purposes of this exercise, it has been assumed that **no *in vivo* animal data exist on the ingredient**
- Focus on **systemic toxicity**
- **Stand-alone illustration of how to assess systemic toxicity effects** (not including genetic toxicity) **using NAMs**

Overall approach for Benzophenone-4 (BP-4)



[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**

Daily use of sunscreen lotion UV-filter*:

- **Amount of sunscreen applied = 18 g/day divided into two applications of 9g (SCCS recommendation)**
- **Concentration in the finished product = 5% (as acid)**

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***Note: to model internal exposures further assumptions need to be made – Module 1**

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

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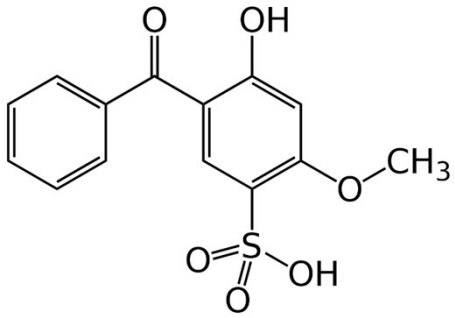
Gathering information: Alerts from *in silico* tools

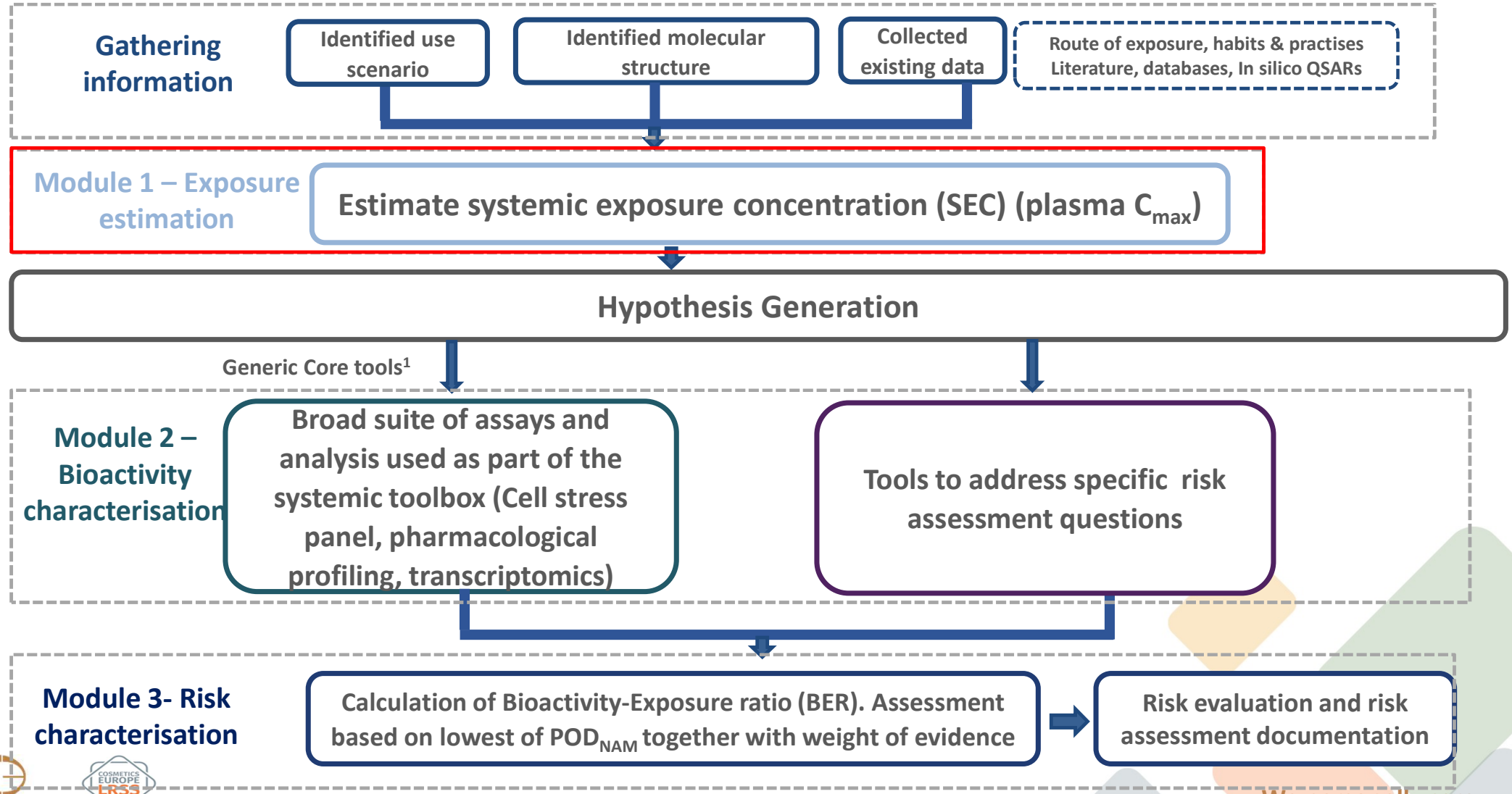
- **Benzophenone-4 did not trigger many alerts within the tools used**. The most common alert across the tools was for skin sensitisation, or protein binding as an indication of skin sensitisation, in the DEREK, TIMES and OECD Toolbox outputs.
- no alerts for DNA binding, non-DART toxicant, no androgen 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)

Follow up with *in vitro* assays to confirm whether or not BP-4 binds to estrogen receptor and other endocrine related endpoints – CALUX EATS estrogenic, androgenic, thyroidogenic and steroidogenesis

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

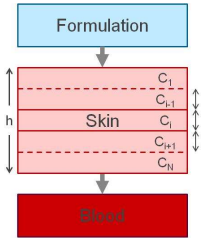
External dose

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

ADME parameters

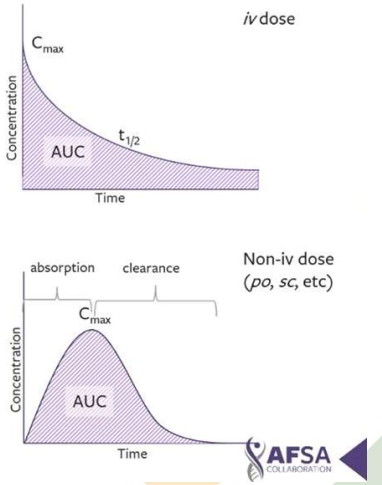
Absorption
Distribution
Metabolism
Elimination

- 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/

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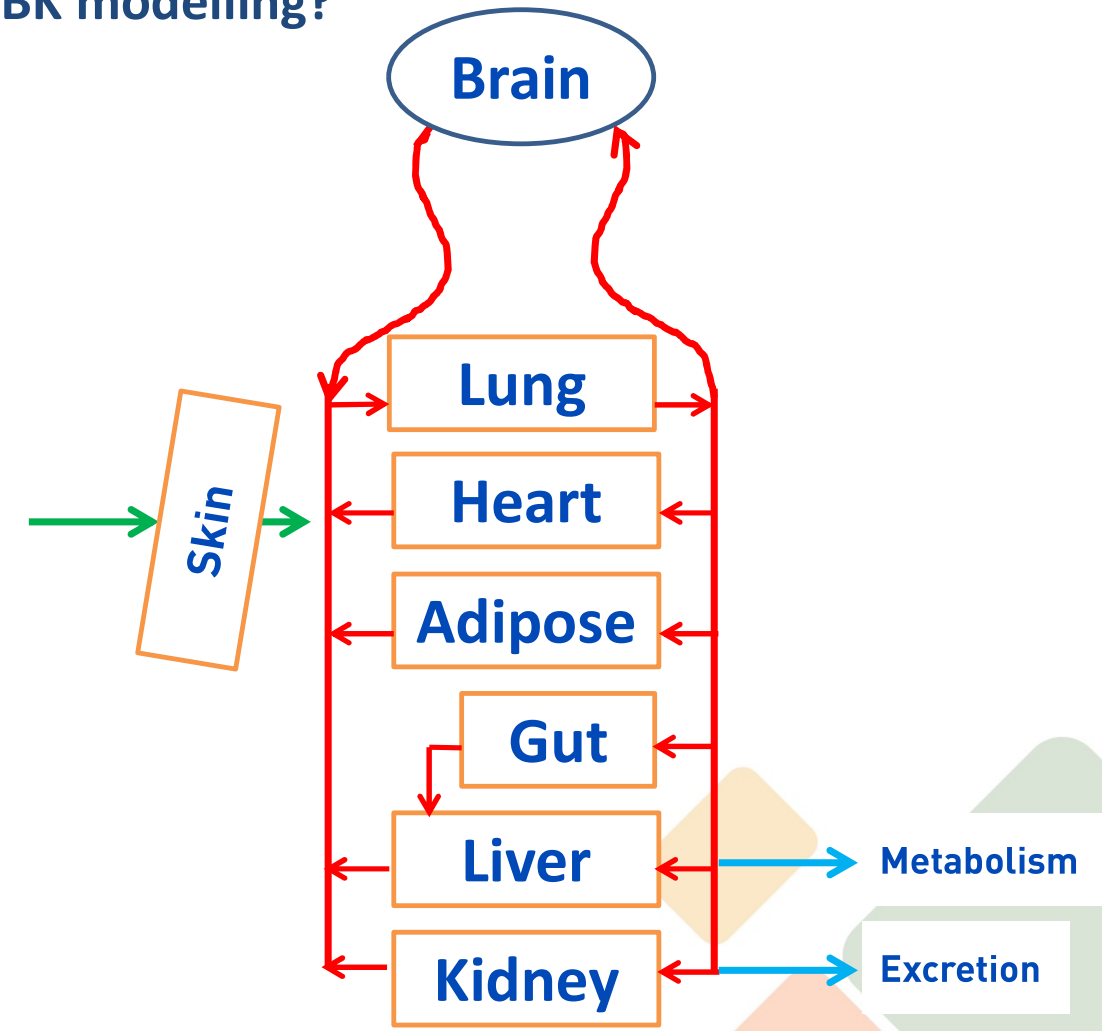
Module 1: Exposure assessment: What is PBK modelling?

- 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



Links to training materials on PBK modelling:

NURA Dynamic discussions: https://pcrm.widen.net/view/video/xr5ojwu8vo/Session2-DyNAMic-Discussions-2023?x.share=true&x.portal_shortcode_generated=a7lwj1xi&x.app=portals
AFSA: https://youtu.be/UGKEMS6DPRo

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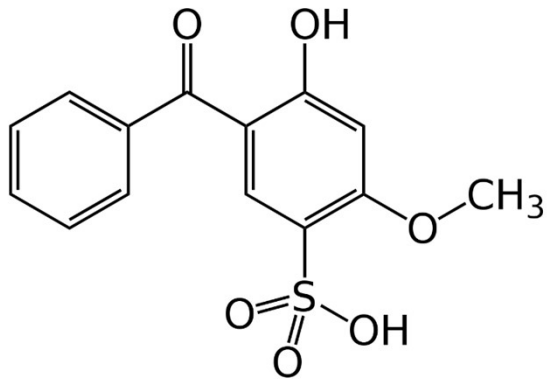
PBK modelling inputs– Exposure scenario and target individual/population

Exposure scenario

- 5% in Sunscreen product,
- 18g/day, two times, 9g/application,
- On body and face 17500cm2 (total body area)
- each day applies the first dose (9g) at 9 am and the second dose (9g) at 2 pm following a meal (fed condition) and this individual takes a shower each morning at 7 am.



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.

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PK-SIM®

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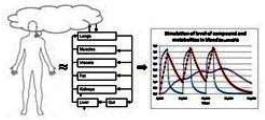
Moxon et al. 2020. Toxicology in Vitro, Volume 63, 104746.



SIMCYP
real solutions from virtual populations

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PBK modelling inputs – ADME data generation

- **In silico tools exists to predict ADME properties from structure (ADMET predictor withing GastroPlus)**
- **The most important ADME properties were generated through in vitro testing:**

  - ***Dermal absorption*:** used to derive kinetic parameters for chemical partitioning in the skin layers and absorption through systemic circulation (OECD TG428). Generated in an *ex vivo* human skin system and using a representative oil/water formulation containing 5% BP4. *BP-4 was found to primarily remain in the vehicle formulation on the skin surface*
  - ***Blood to plasma ratio*:** determines the concentration of the drug in whole blood compared to plasma and provides an indication of chemical binding to erythrocytes. *No binding activity for RBCs*
  - ***Plasma protein binding***: the degree of binding determines the free available concentration of the chemical in plasma. *High binding to human plasma proteins (98.4%)*
  - ***Metabolic stability*:** evaluated using different methods (suspension and plated primary hepatocytes) and it is used to understand the route of elimination of a chemical and derive values for intrinsic hepatic clearance and half-life. *BP-4 stable in primary human hepatocytes.*

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

PBK modelling inputs – ADME results

	Source
Molecular weight	308.31 g/mol
Log P	ADMET predictor
pKa	ADMET predictor
Fraction unbound in plasma (f_{up})	**Measured**
Blood: plasma ratio	**Measured**
Hepatic intrinsic clearance (L/h)	**Measured**, suspension and plated primary human hepatocyte assay, Pharmacelsus
ECCS classification	*Varma et al., 2015*
Renal excretion	GFR*Fup
Dermal absorption parameters: Partition coefficient and diffusivity in skin layers	**Measured**, Eurofins, *Ex vivo* skin penetration study designed according to *Davis et al. 2011* meeting OECD TG 428 and SCCS guidance

Main observations:

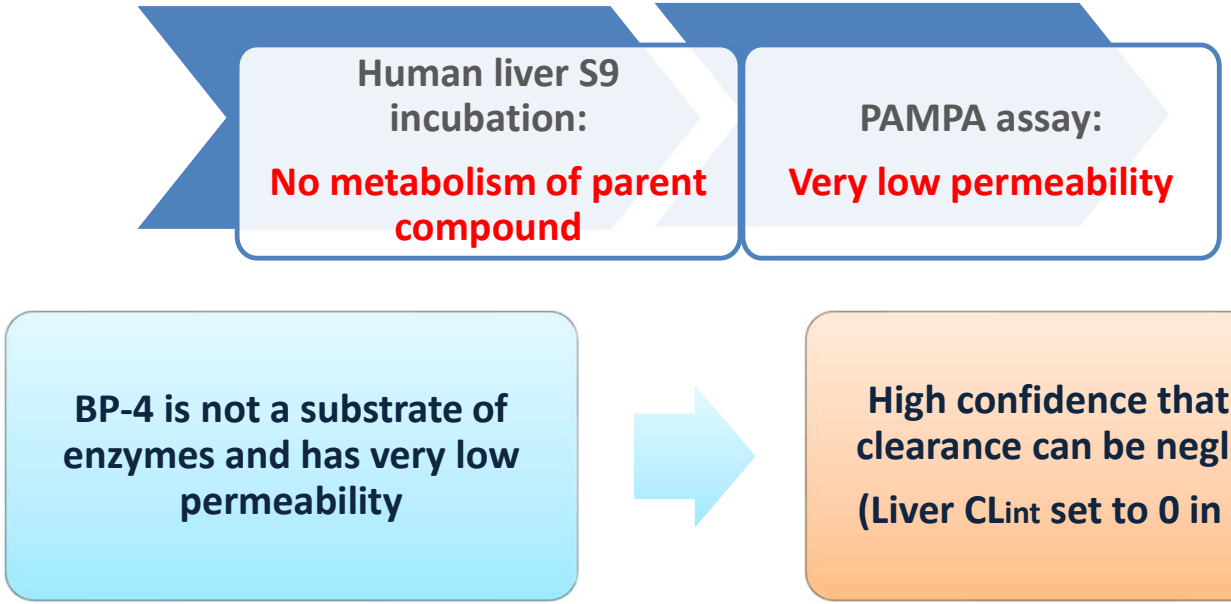
- Very low skin penetration
- **BP-4 stable in human hepatocytes**. Hepatic intrinsic clearance <2.5L/h (Below LOQ)

Conclusion: Clarify hepatic clearance and understand the route of elimination

Clarify the hepatic clearance - two hypotheses:

1) **Benzophenone-4 is not a substrate of CYP enzymes – need to confirm with a second assay using S9 fraction**
    - **Note, BP-4 is a hydrophilic compound already**
2) **Benzophenone-4 has low membrane permeability–** Parallel artificial membrane permeability (PAMPA) assay that measures passive permeation across a lipid layer

Follow up assays



Next steps to understand the route of elimination… Understanding chemical organ distribution and renal clearance

In silico predictions:

- BP-4 is an anion sulphonate
- Likely to be a substrate of Organic anion transporters (OATs)
- **Question: Is BP-4 actively transported by active transporters in kidney?**

	Transporters	Uptake of efflux?	Substrate?	Molecular Mass (Dalton)	Vmax (mg TA/mg transporter/s)	Km (μM)
Uptake Transporter Substrate Assays	OAT1	Uptake	**Yes**	61816.3	0.01408	8.89
	OAT2	Uptake	**Yes**	60025.7	0.01639	146.0
	OAT3	Uptake	**Yes**	59856.2	0.00398	13.47
	OCT2	Uptake	No			-
	MATE1	Efflux	No			-
	MATE2-K	Efflux	No			-
Vesicular Transport Substrate Assays	MRP2	Efflux	No			-
	MRP4	Efflux	**Yes**	149526.8	0.0026	48.54
	MDR1/Pg-p	Efflux	No			-
	BCRP	Efflux	**Yes**	72314.2	0.00359	74.68

Assay:

Transporter studies in transfected kidney cells in two different assays (uptake assay and efflux assay)

Results:

- **Substrate of the influx transporters, OAT1, OAT2 and OAT3 and a substrate of the efflux transporters, BCRP and MRP4.**
- **Vmax and Km determined for each transporter**
- **actively transported by active transporters in human PTC**

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https://doi.org/10.1002/jcph.702

Next steps to understand the route of elimination… Understanding chemical organ distribution and renal clearance

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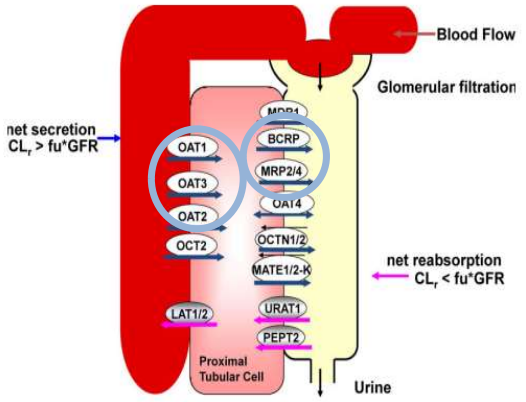

Figure 1. Mechanism of drug elimination and major transporters in the kidney. Drug elimination in the kidney is through glomerular filtration, secretion, and reabsorption process. Major transporters localized in the proximal tubule cells are depicted. The blue arrows indicate secretion, and the pink arrows indicate reabsorption.

https://doi.org/10.1002/jcph.702

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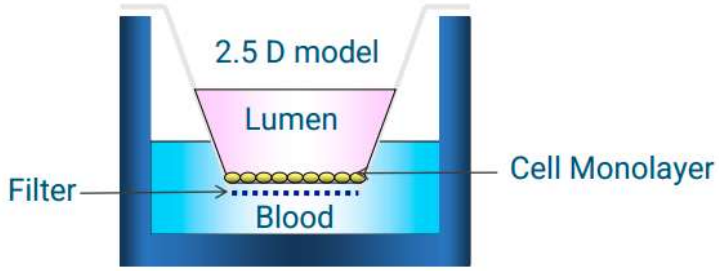
- **Vmax and Km determined for each transporter**
- **actively transported by active transporters in human PTC**

Next steps to understand the route of elimination… Understanding chemical organ distribution and renal clearance

Question:

- What is the overall balance between secretion and reabsorption?
- Accumulation in proximal tubular cells?



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

Assay:

Bidirectional permeability of BP-4 in **freshly isolated kidney proximal tubule cells monolayer in transwell system (aProximate™).**

Results:

- transport in both directions is equally efficient leading to no net movement or intracellular accumulation
- GFR*Fup was used to calculate renal excretion of benzophenone-4, accounting for filtration only to be conservative

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Newcells aProximate™ platform

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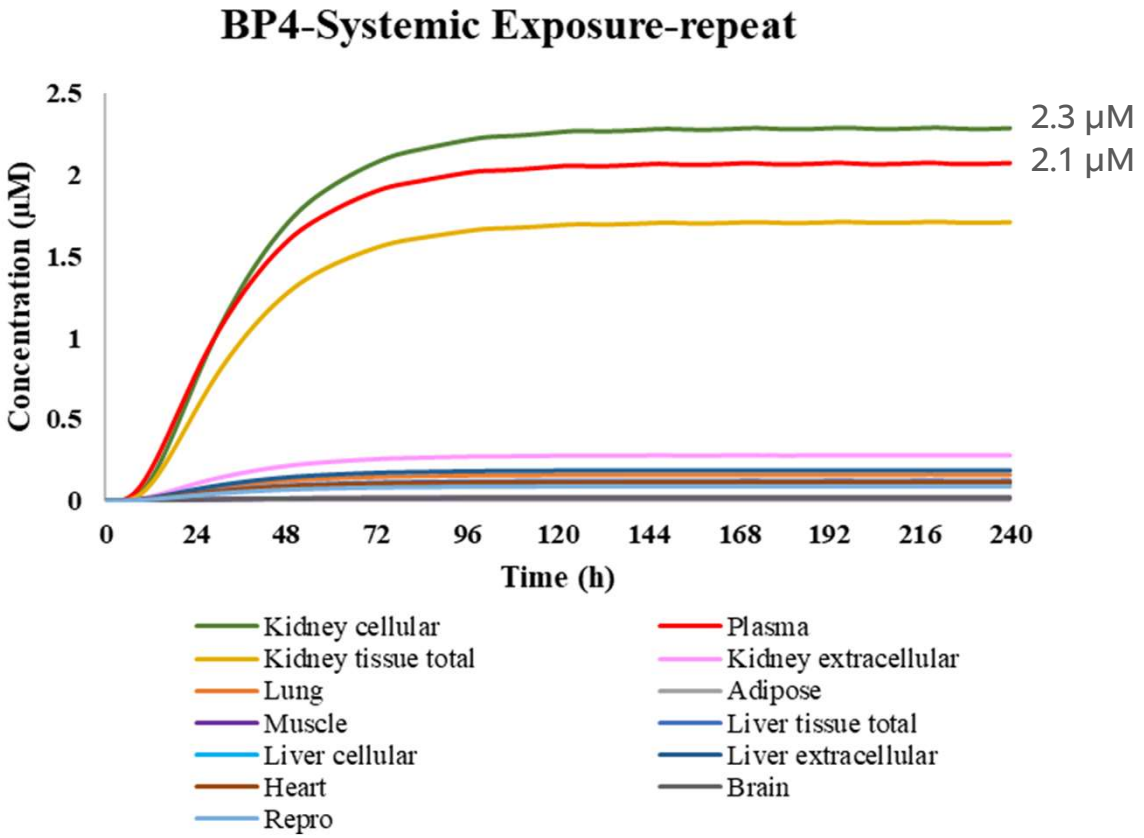
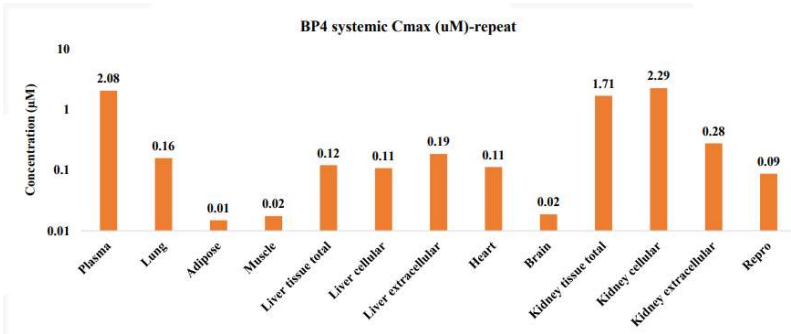
Internal concentration: Deterministic PBK model simulation of C_{max} for an adult female (30 years old, 60 kg)



PK parameter	Value
Bioavailability (%)	0.4
CL_{renal} (L/h)	0.11
Plasma C_{max} (μM)	2.08
AUC_{24h} (ug-h/mL)	1.94
Volumes of distribution at steady state (L)	8.577
$t_{1/2}$ (h)	54.3



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:

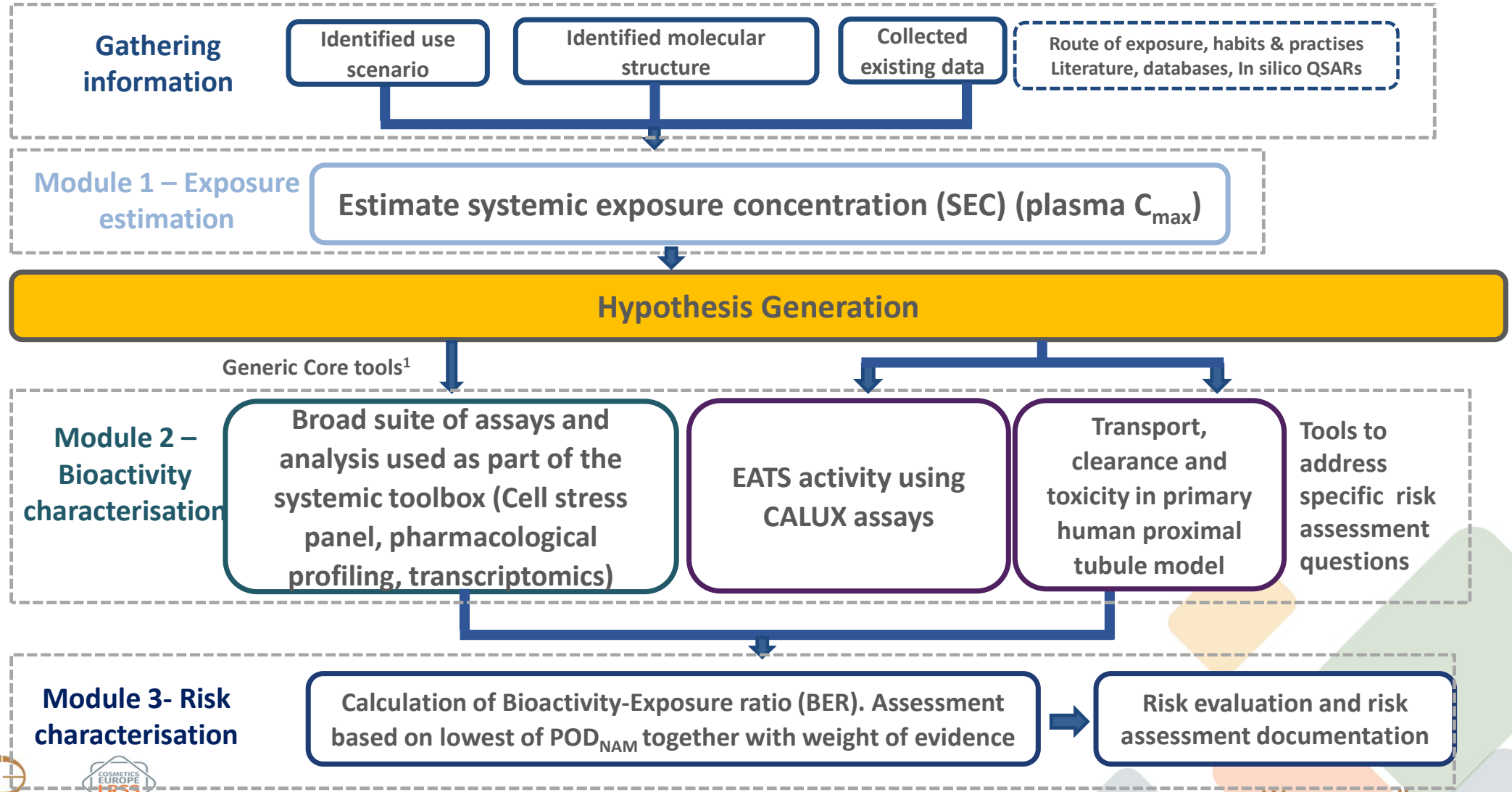
- Overall, upon dermal absorption only a small amount of BP-4 enters systemic circulation, after which BP-4 remains unchanged due to negligible liver clearance.
- It has low tissue distribution due to low partitioning and limited passive diffusion of cell membranes (charged at physiological pH).
- It 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.
- Successive doses result in accumulating concentrations of BP-4 in the body until a steady state is reached at around 100h when there is an equilibrium reached between the low absorption and low excretion into the urine.

Breakout discussion

www.slido.com
enter code
#ASCCT

1. How informative are the *in silico* prediction results?
2. How confident are you from the clarification of the hepatic clearance data?
3. How confident are you from the clarification of the route of elimination?
4. How confident are you in the deterministic predicted values of plasma Cmax?
5. How would these exposure results inform your next steps in the risk assessment?
6. How would you address the remaining uncertainty in this predicted value in the risk assessment? (i.e. What other information would you like?)

Overall approach for Benzophenone-4 (BP-4)



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

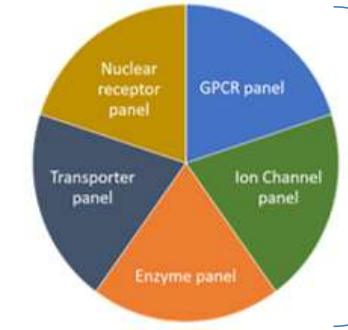
Hypothesis Generation

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 nontargeted biological screen

In vitro pharmacological profiling



~79 targets

eurofins | Cerep


PERSPECTIVES

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

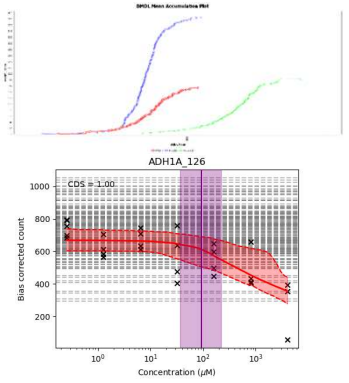
Bowes et al. 2012. Nat Rev Drug Discov 11(12): 909-22

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

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

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

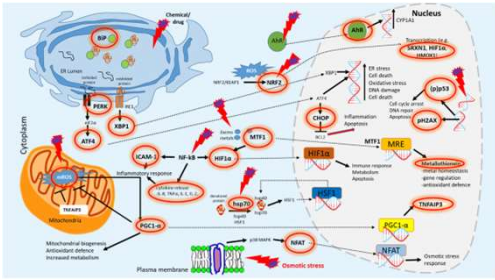


Image kindly provided by Paul Walker (Cyprotex)

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

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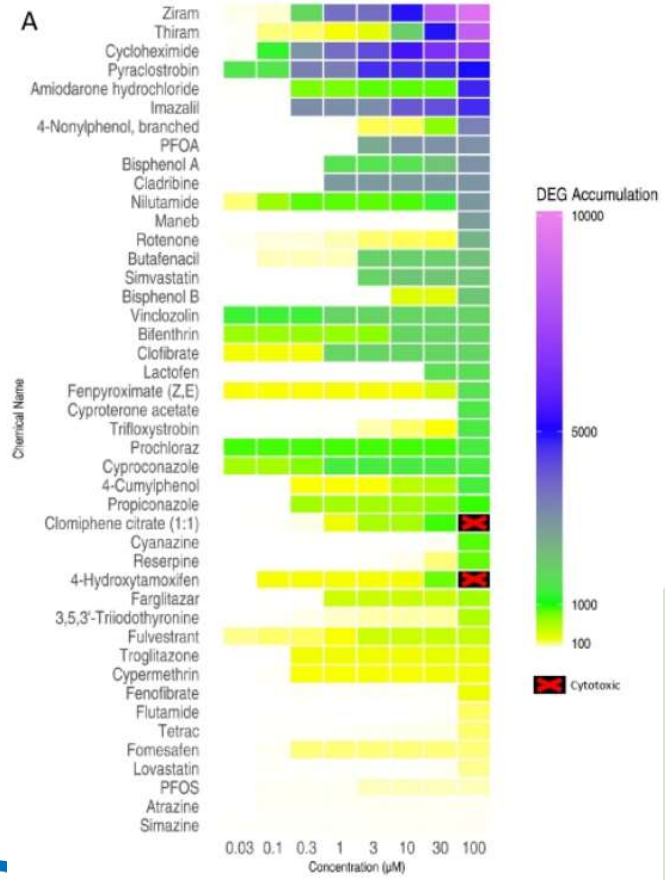
High Throughput Transcriptomics (HTTr) applied as a broad nontargeted biological screen

- 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™))

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



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Harrill et al., 2021. Tox Sci 181:1, Pages 68–89

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Cell stress panel- 10 stress pathways responsible for cell homeostasis

Objective:

To characterize **non-specific biological activity** which is not mediated via a specific protein/receptor interaction - covering ~10 cell stress pathways using high content imaging analysis

cyprotex AN EVOTEC COMPANY

- **~10 Stress Pathways: 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
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Research article

FEATURED

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

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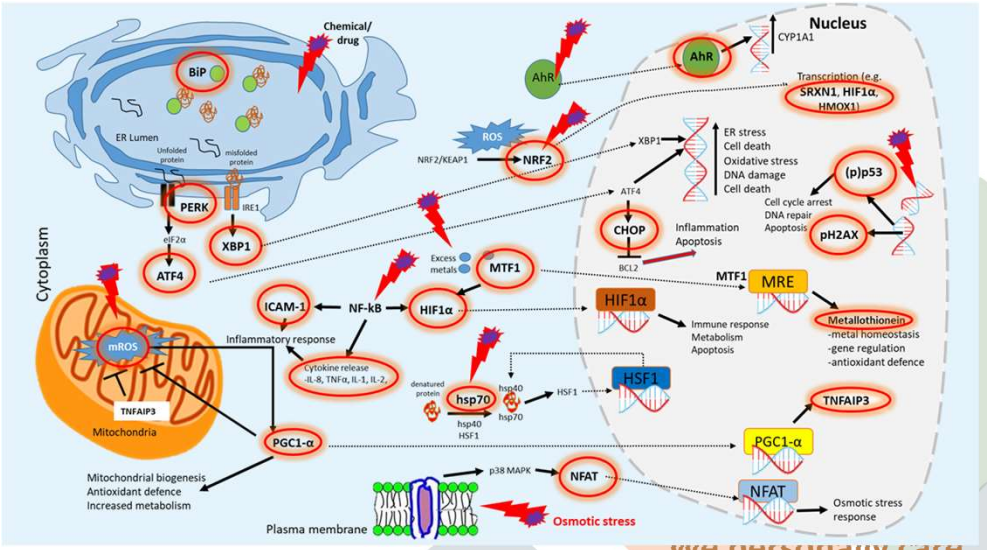

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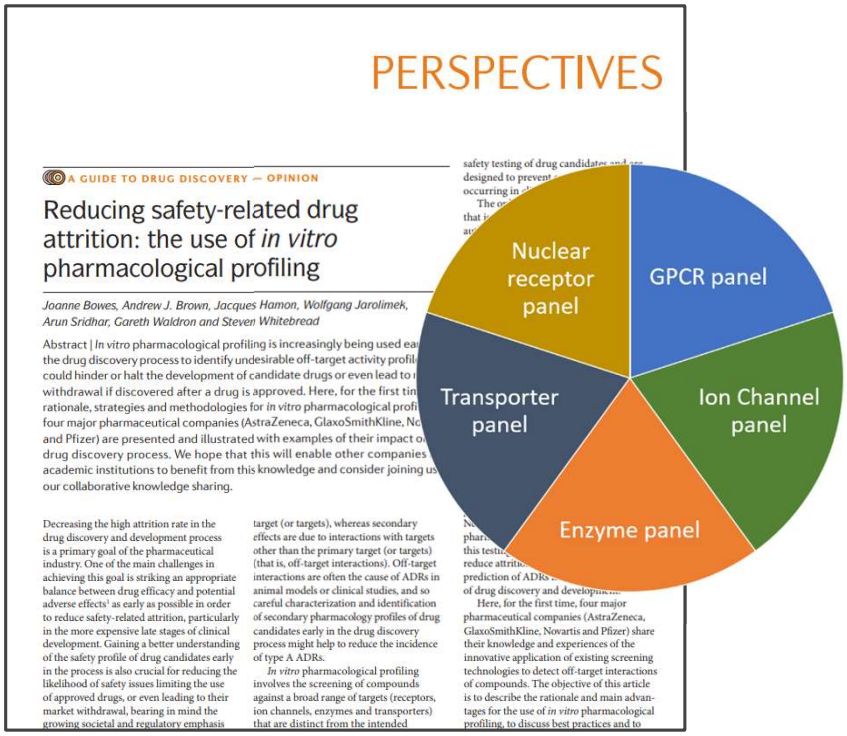
[1]Middleton et al. (2022) *Toxicol Sci* (https://doi.org/10.1093/toxsci/kfac068)

In vitro pharmacological profiling- currently 79 targets



- 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]

Targets (gene)	Hit rate*		Main organ class or system	Effects	
	Binding	Functional or enzymatic		Agonism or activation	Antagonism or inhibition
G protein-coupled receptors					
Adenosine receptor A_{2A} (*ADORA2A*)	High	Low (agonist)	CVS, CNS	Coronary vasodilation; ↓ in BP and reflex; ↑ in HR; ↓ in platelet aggregation and leukocyte activation; ↓ in locomotor activity; sleep induction	Potential for stimulation of platelet aggregation; ↑ in BP; nervousness (tremors, agitation); arousal; insomnia
α_{1A}-adrenergic receptor (*ADRA1A*)	High	Low (agonist); high (antagonist)	CVS, GI, CNS	Smooth muscle contraction; ↑ in BP; cardiac positive ionotropy; potential for arrhythmia; mydriasis; ↓ in insulin release	↓ in smooth muscle tone; orthostatic hypotension and ↑ in HR; dizziness; impact on various aspects of sexual function
α_{2A}-adrenergic receptor (*ADRA2A*)	High	Low (agonist); medium (antagonist)	CVS, CNS	↓ in noradrenaline release and sympathetic neurotransmission; ↓ in BP; ↓ in HR; mydriasis; sedation	↑ in GI motility; ↑ in insulin secretion
β_1-adrenergic receptor (*ADRB1*)	Medium	NA	CVS, GI	↑ in HR; ↑ in cardiac contractility; electrolyte disturbances; ↑ in renin release; relaxation of colon and oesophagus; lipolysis	↓ in BP; ↓ in HR; ↓ in CO
β_2-adrenergic receptor (*ADRB2*)‡	High	Medium (agonist); medium (antagonist)	Pulmonary, CVS	↑ in HR; bronchodilation; peripheral vasodilation and skeletal muscle tremor; ↑ in glycogenolysis and glucagon release	↓ in BP

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

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: Tools to address specific risk assessment questions

2. In silico prediction for estrogen binding

EATS activity: estrogenic, androgenic, thyroidogenic and steroidogenesis

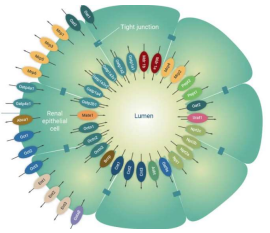
- **CALUX bioassays to measure transcriptional activation** and **binding assays: TTR-TRβ- and hTPO**
- **U2-OS** incorporating the firefly **luciferase reporter gene** coupled to Responsive Elements (REs)
- **12 concentrations**. Calculation of AC50, LOEC and NOEC

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

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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 (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 uM
- ~83 targets compiled by Cosmetics Europe Safety pharmacology WG
- **No hits**

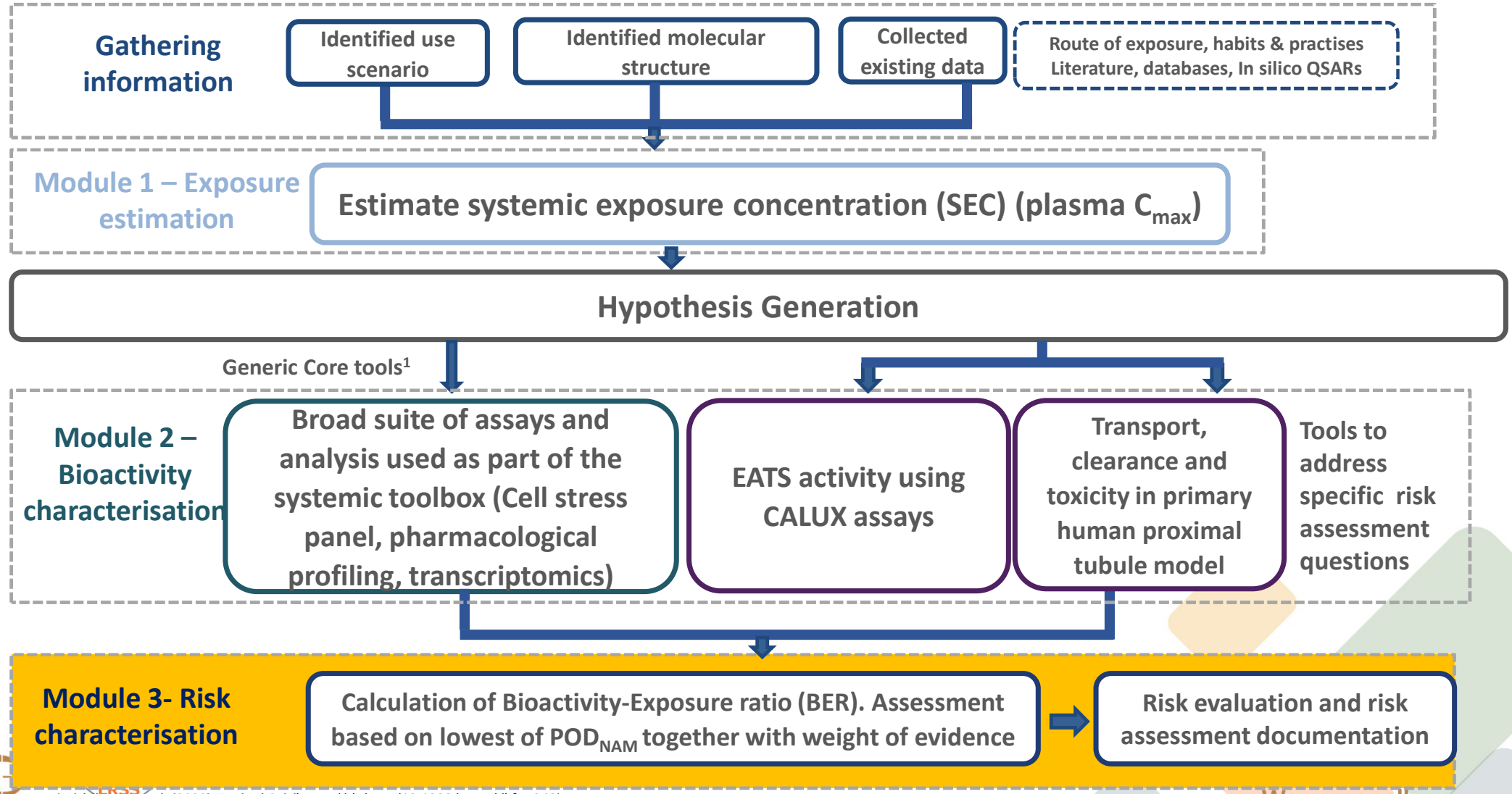
Calux assays

- 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)

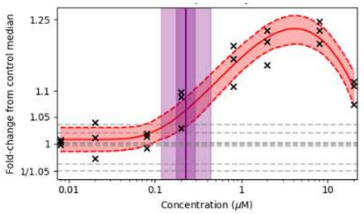
Overall approach for Benzophenone-4 (BP-4)



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

Calculation of the Bioactivity Exposure Ratio

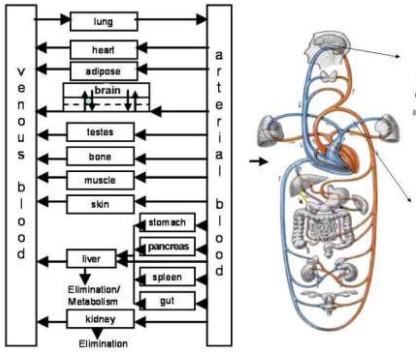
Point of departure (POD) derived from concentration-response data



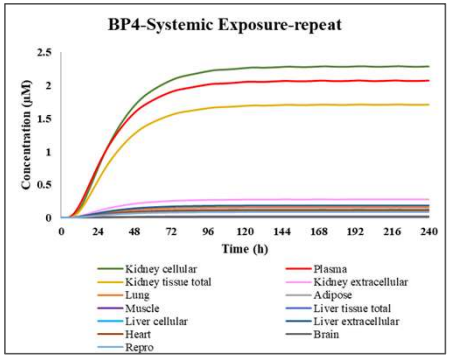
Systemic toolbox of assays (NAMs) which cover a broad biological space – **measurements of bioactivity**

- 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

But…from a quantitative perspective… How do we define an acceptable BER to conclude an exposure to a give chemical is low risk?

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

1. **The in vitro measures of bioactivity provide appropriate biological coverage**
2. **There is confidence that the test systems are at least as sensitive to perturbation as human cells *in vivo***
3. **The exposure estimate is conservative for the exposed population**

Bioactivity: exposure ratio calculation

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

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.

Breakout discussion 2

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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. How would you address the remaining uncertainty in the risk assessment? (i.e. What other information would you like?)

One way at looking at the uncertainties– Qualitative assessment

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

Areas

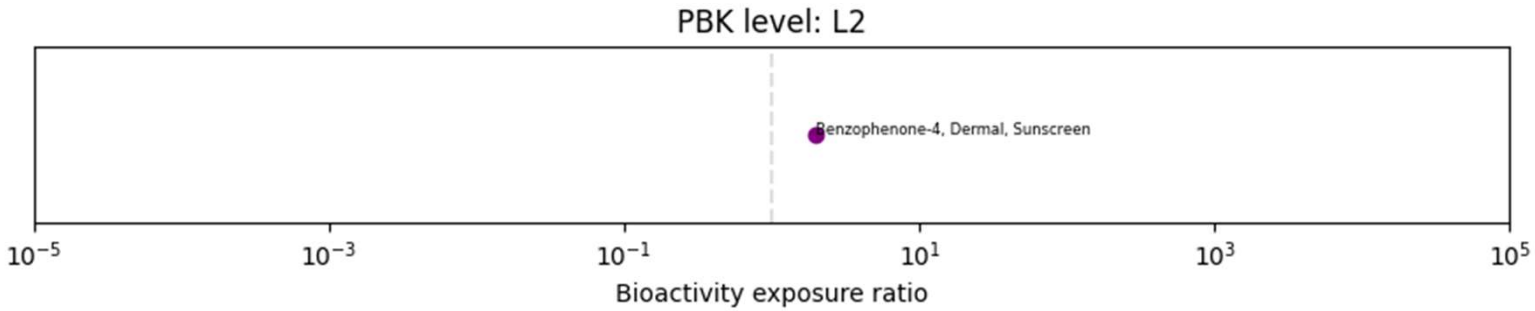
- Consumer exposure (applied dose)
- Identification of metabolites
- Consumer exposure (Internal dose)
- Range of biomarkers assessed
- Use of short-term tests *in vitro* to inform about risks of long-term human exposure
- Point of departure selection

Similar approach to OECD (2021): IATA for Phenoxyethanol

One way at looking at the uncertainties– Qualitative assessment - Example

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

Let’s have a look at the deterministic BER using the best PBK model (BER=2)



Question 1. Given all this information would you conclude, low risk, uncertain risk or high risk?

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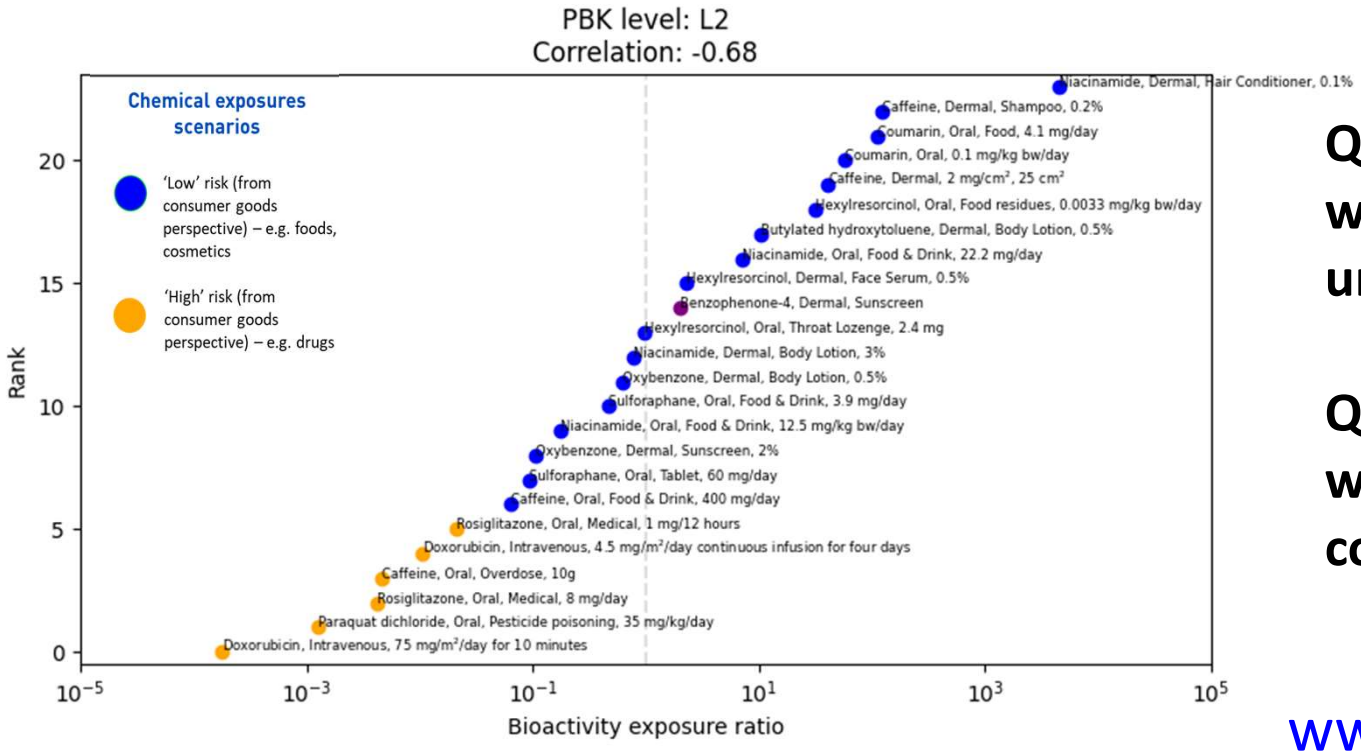
What if the same approach was applied to 10 other chemicals with varying risk classifications



Q2. Given this new information would you conclude low risk, uncertain risk or high risk?

Q3. What other information would you need to improve your confidence in a low risk outcome?

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Note: Low risk is different than low toxicity; it is all about integrating exposure.

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Conclusions & reflections

- **Showcased a range of in silico and in vitro NAMs that can be used for safety decision making for systemic toxicity**
- **The method 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**

Repeated dose toxicity in rats in combination with Reproductive/Developmental toxicity study: via oral route (reliable without restriction)
Remarks on the results: no effects observed, large MoS
NOAEL >= 1 250 mg/kg bw/day (actual dose received), source https://echa.europa.eu/ (reminder: our exposure scenario was 15 mg/kg bw/day)

Acknowledgments

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Alistair Middleton

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

ADDITIONAL SLIDES

Strategies in addressing uncertainty in PBK estimation

*Middleton, A.M., et al., Are Non-animal Systemic Safety Assessments Protective? A Toolbox and Workflow. Toxicological Sciences, 2022. **189**(1): p. 124-147.*

Deterministic PBK modelling

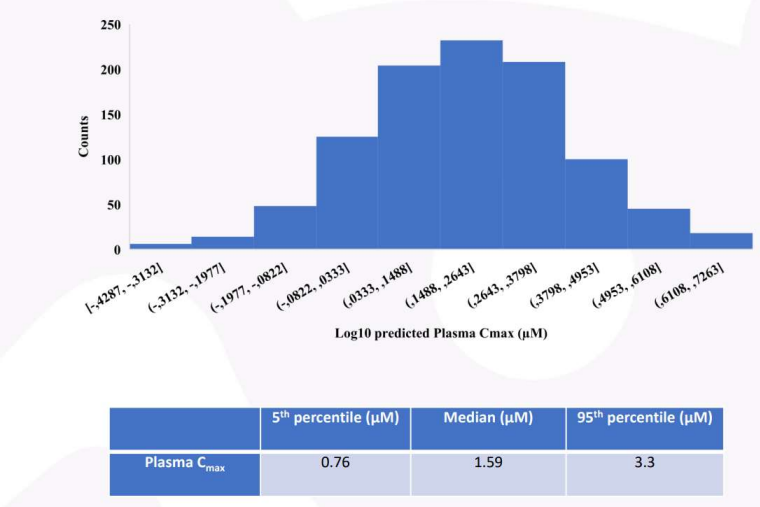
Point estimate values for input parameters → Model

Individual modelled (30 year-old 60 kg female, European)

Probabilistic population PBK modelling

Parameter Uncertainty ('informed' distribution for the most sensitive parameters) → Model

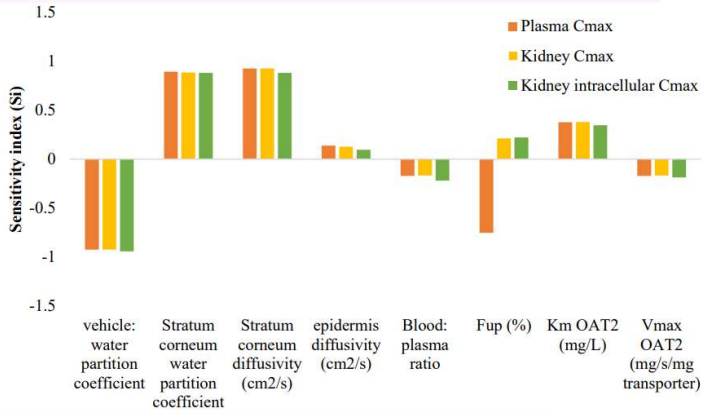
Population Variability



	5th percentile (µM)	Median (µM)	95th percentile (µM)
Plasma C_{max}	0.76	1.59	3.3

distributions for parameters used in uncertainty analysis and probabilistic PBK simulations

Parameter	Mean	cv%		Distribution type	Lower Limit	Upper Limit
Fup	1.574	37.21	In vivo variability + In vitro standard deviation	lognormal	0.6095	4.0651
kidney volume	324.3	30		normal	32.4348	616.261
Liver volume	1416.1	30	Table 2 from Clewell and Clewell III, 2008	normal	141.612	2690.63
liver plasma partition coefficient	0.09	20		lognormal	0.05209	0.15555
kidney plasma partition coefficient	0.135	20		lognormal	0.07795	0.23277
OAT2 expression in liver	3.50E-03	56.63	Literature review	lognormal	0.00091	0.01345
Km MRP4	1.5	25		lognormal	0.768	2.92969
Vmax MRP4	2.60E-03	25		lognormal	0.00133	0.00508
Km OAT2	4.5	25		lognormal	2.304	8.78906
vehicle: water partition coefficient	120	25	In vitro standard deviation	lognormal	64.486	234.38
Stratum corneum water partition coefficient	1	70		lognormal	0.2035	4.913
Stratum corneum diffusivity	2.00E-11	70		lognormal	4.07E-12	9.83E-11
epidermis diffusivity	6.00E-10	130		lognormal	4.93E-11	7.30E-09



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Model

Population Variability

Probabilistic population PBK+ CMED modelling

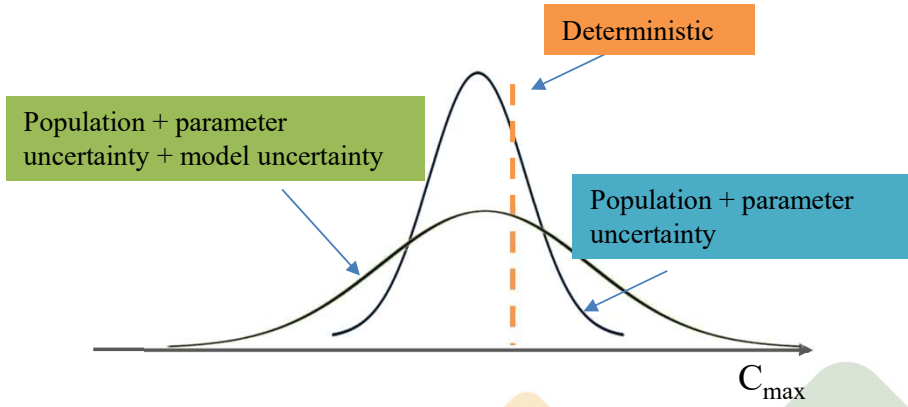
Parameter Uncertainty

Model Uncertainty

Model

Population Variability

Predicted C_{max} based on different approaches characterising uncertainty

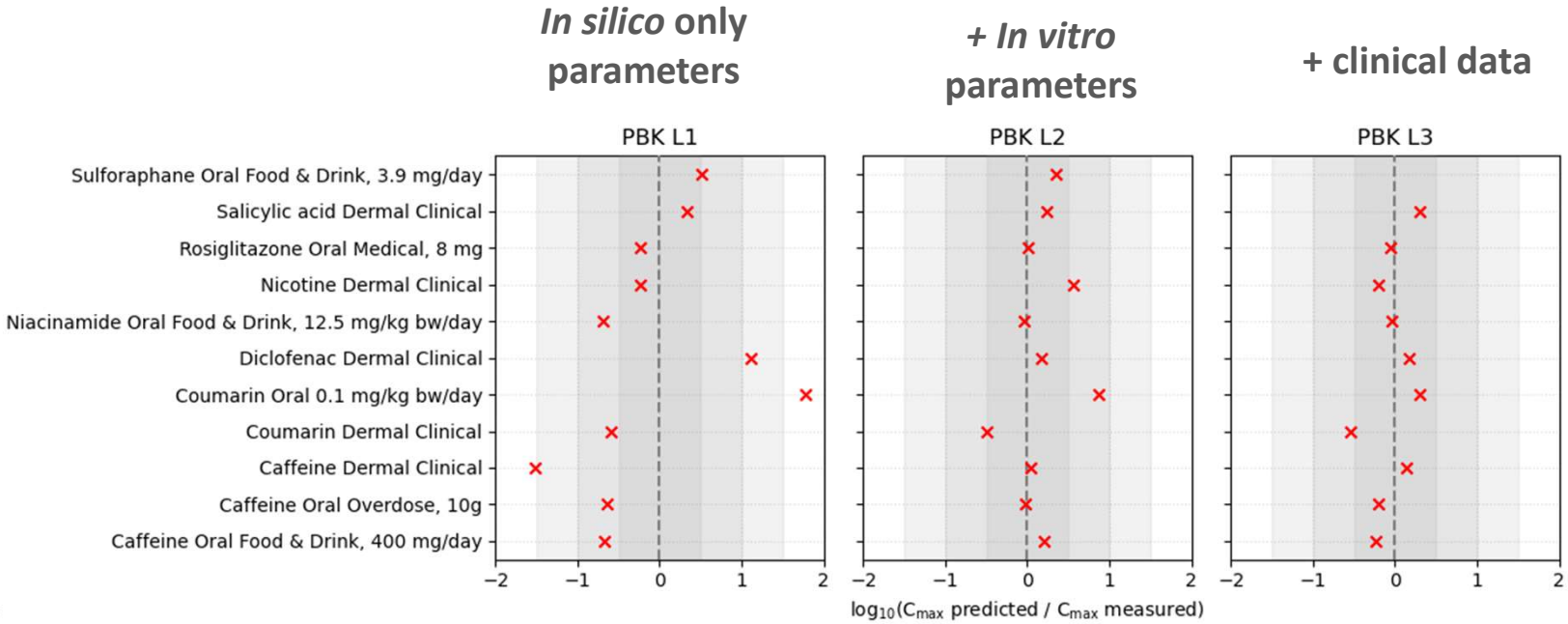


Deterministic PBK model for adult female 60 kg	Distribution of C_{max} within the in vivo population estimated by combining CMED model and GastroPlus™ population simulation (µM) (green curve)	
Plasma C_{max} point estimate	Median (95% interval)	95th percentile
2.1	1.3 (0.11, 15)	9.8

Probabilistic PBK modelling + CMED model to account for population, parameter and model uncertainty

To account unknown-unknows e.g. model uncertainty

- C_{max} Error Distribution (CMED): A complementary approach to characterise PBK prediction uncertainty as published in Middleton *et al.* 2022.
- This model seeks to quantify the error distribution of estimates of plasma C_{max} by looking at the difference between PBK predictions of C_{max} and existing measured values in human clinicals for several exposure scenarios.
- This model can be used to estimate the distribution of the possible prediction errors for future chemical and exposure scenario.



and Workflow. Toxicological Sciences, 2022. 189(1). p. 124-147.

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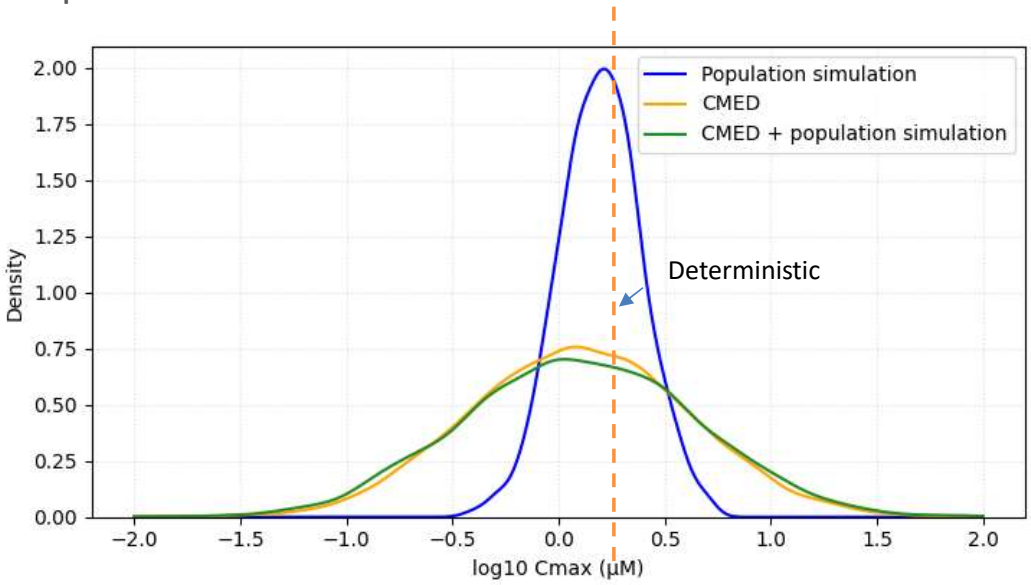

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Confidence level

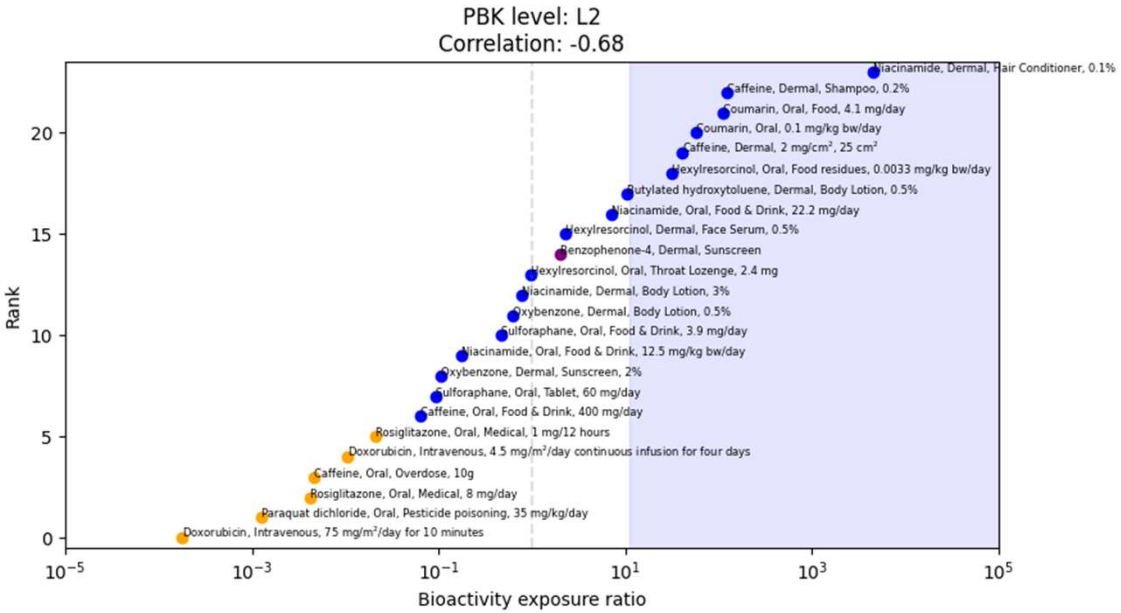
WHO questions for assessing the level of confidence in the BP-4 PBK modeling

Model evaluation aspect	level of confidence (towards the accuracy)	level of confidence (towards the conservatism)
Do the model structure and parameters have a reasonable biological basis?	High	**High**
How well does the PBK model reproduce the chemical-specific PK data under various experimental or exposure conditions?	Low	**High**
How reliable is the PBK model with regard to its predictions of dose metrics relevant to risk assessment?	High	**High**

Conclusions

- ✓ The stepwise way of data generation and refinement, using relevant and robust approaches for parameter determination, support the reliability of input parameters and provide a sound biological basis for the model structure.
- ✓ Although human clinical data are not available for validation, the sensitivity and uncertainty analyses and the probabilistic modelling performed provided assurance that the predictions are fit for purpose and provides conservative estimates of human systemic exposure.

What if the same approach was applied to 10 other chemicals with varying risk classifications



Question 1. Given this new information would you conclude low risk, uncertain risk or high risk?

Question2. If your decision changed, what changed your mind?

Question3. What other information would you need to improve your confidence in a low risk outcome?

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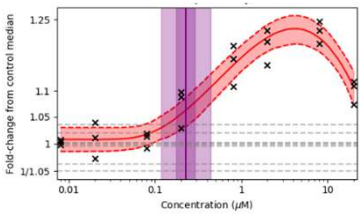
Conclusions & reflections

- 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

Approach to this Next Generation Risk Assessment

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



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

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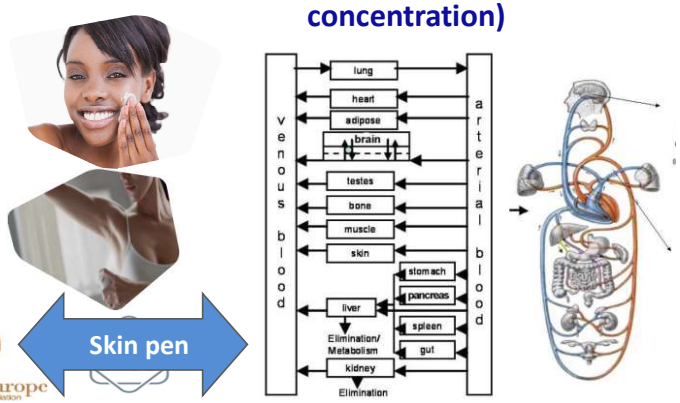
- Others

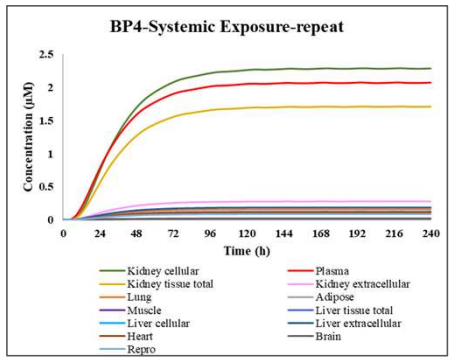
Calculation of Bioactivity exposure ratio (BER)

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

Exposure models (PBK, free/total concentration)



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



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